[PROC. ROY. SOC. VICTORIA, **54** (N.S.), PT. II., 1942.]

ART. VIII.—*Studies on Soil Conditions in Relation to Root-rot of Cereals.*

By C. R. MILLIKAN, M.Agr.Sc.

[Read 11th September, 1941; issued separately, 31st August, 1942.]

Contents.

In Victoria, the effect of mineral nutrition on the severity of eelworm (*Heterodera schachtii* and *Pratylenchus pratensis*) and root-rot disease of cereals was first studied in 1936. Small applications of zinc sulphate to Wimmera Black fallow soil were found to promote a marked increase in growth and yield of wheat and oats, while the severity of the above diseases was reduced (Millikan 1938). Subsequent investigations have been directed at determining the effects of other minerals on the growth of cereals grown in Wimmera soil, and the nature of some of the factors concerned in promoting the responsiveness of the plants to applications of such minerals. The significance of these factors in relation to the apparent severity of root-rot disease has also been studied. The results of this work are set out hereunder.

Nutritional Studies with Root-rot Fungi.

While Steinberg (1938), Blank (1939, 1941), Foster (1939) and numerous other workers have shown that the need for minute quantities of various heavy metals is a phenomenon widespread amongst fungi generally, there is at present little information available regarding the heavy metal requirements of specific cereal root-rot fungi.

Experiments have therefore been conducted to determine whether certain elements which are known to be essential for the growth of higher plants are also essential for the normal development of some of the more common root-rot fungi occurring in Wimmera soil.

Method.

Numerous preliminary experiments using *Helminthosporium sativum* and *Fusarium culmorum* were made to evolve a satisfactory synthetic nutrient solution which would encourage the normal growth of these fungi. Various solutions were tried including that used by Steinberg (1936) for *Aspergillus niger.*

Some of the solutions, those containing ammonium nitrogen in particular, did not encourage normal sporulation and pigmentation. Starch and calcium chloride improved the growth of the fungi, notably in the case of *F. culmorum.* In this regard, Young and Bennett (1922) have reported that calcium stimulated the growth of a number of parasitic fungi grown in synthetic culture media, while Rogers (1938) obtained an appreciable increase in the growth of *Phymatotrichum omnivorum* by the addition of starch to the media.

The addition of amino nitrogen and vitamins to the solution also stimulated growth in some instances. (Table 1.)

As regards the heavy metals, several experiments were conducted to determine suitable concentrations. The following summarises the principal results obtained:—

A concentration of five parts per million of copper was found to depress the growth of *H. sativum* and *C. ramosa,* although *F. culmorum* appeared to tolerate this amount. On the other hand, all fungi appeared to tolerate concentrations of five parts per million of either zinc or iron. Two parts per million of molybdenum, cobalt, boron, or nickel depressed the growth of all the above fungi.

The composition of the final solution adopted was as follows:—

Di-potassium hydrogen phosphate	1.0 grams per litre
Magnesium sulphate	0.5 ,, ,, ,,
Potassium chloride	0.5 ,, ,, ,,
Sodium nitrate	2.0 ,, ,, ,,
Sucrose	30.0 ,, ,, ,,
Starch	10.0 ,, ,, ,,
Calcium chloride	0.5 ,, ,, ,,
Asparagine	0.5 ,, ,, ,,
Glycine	0.2 ,, ,, ,,
Thiamin (Vitamin B_1)	5 parts per million
l-ascorbic acid (Vitamin C)	5 ,, ,, ,,
Nicotinic acid	5 ,, ,, ,,
Iron (as $FeSO_4 . 7H_2O$)	1 ,, ,, ,,
Zinc (as $ZnSO_4 . 7H_2O$)	1 ,, ,, ,,
Manganese (as $MnSO_4 . 7H_2O$)	0.5 ,, ,, ,,
Copper (as $CuSO_4 . 5H_2O$)	0.1 ,, ,, ,,
Double distilled water	1,000 ml.

Amino nitrogen was omitted for studies with *Helminthosporium sativum* and vitamins for studies with *Fusarium culmorum* (see Table 1). Pure synthetic vitamins were used. These were added to the solution after the purification process described below to prevent dissociation by heat.

The double distilled water was produced by a Pyrex glass still, and all chemicals were of analytical quality. Before the addition of the heavy metals and vitamins, the solutions were purified by autoclaving with 15 grams of calcium carbonate per litre at one atmosphere pressure for twenty minutes and then filtering (Steinberg 1935). After this treatment the heavy metal contamination of the solution was tested by the dithizone method described by Stout and Arnon (1939).

The results of each of these tests showed that the total contents in each of the solutions of all the metals which react with dithizone was less than 0·02 parts per million.

The solutions were then distributed to pyrex glass erlenmeyer flasks of 200 c.cs. capacity and the heavy metals in solution were added by means of a pipette. All glassware was cleaned with 1 : 1 hydrochloric acid and rinsed in double distilled water before use. After sterilization, 5 c.cs. of a suspension of spores of the appropriate fungus was added to each flask, bringing the final volume of the solution per flask to 50 c.cs. The cultures were then incubated at 25°C. for fourteen days.

After incubation the cultures were filtered, and the mycelial mat was washed with hot water, dried in an oven at 103°C. for three hours and then weighed. Each treatment was replicated three times.

Results.

The comparative effects of amino nitrogen and vitamins on the growth of *F. culmorum, H. sativum* and *C. ramosa* are shown in Table 1.

Table 1.—Effects of Amino Nitrogen and Vitamins on Growth of Cereal Root-rot Fungi in Nutrient Solutions Incubated at 25° C. for Ten Days.

	Fusarium culmorum.		Helminthosporium sativum.		Curvularia ramosa.	
——	Dry Weight of Fungus in Grams.	Per cent of Base Medium.	Dry Weight of Fungus in Grams.	Per cent of Base Medium.	Dry Weight of Fungus in Grams.	Per cent of Base Medium.
Base medium	0·366	100	0·388	100	0·531	100
Base medium + amino nitrogen*	0·553	151	0·352	91	0·664	125
Base medium + vitamins 5 p.p.m.†	0·324	89	0·489	126	0·529	100
Base medium + amino nitrogen + vitamins 1 p.p.m. ..	0·492	134	0·369	95	0·635	120
Base medium + amino nitrogen + vitamins 5 p.p.m. ..	0·449	123	0·382	99	0·606	114
Base medium + amino nitrogen + vitamins 20 p.p.m.	0·520	142	0·362	93	0·655	123
Difference for significance	0·070	19·1	0·043	11·1	0·055	10·4

* Asparagine 0·5 grams and glycine 0·2 grams per litre.
† Thiamin (B_1) l-ascorbic acid (C), and nicotinic acid.

Although the general results of these experiments were in accord with those shown in Table 1, some variation in degree occurred, due probably to the use of different strains of the fungi. It is known that the physiology of such strains may vary. For instance, Pervukhina (1938) found that several different strains of species of *Fusarium* on wheat differed widely in the amount of amino nitrogen accumulated by them. Such differences were correlated with differences in their pathogenicity to wheat.

While amino nitrogen stimulated the growth of *F. culmorum* and *C. ramosa,* it induced marked sectoring and usually a depression of the yield of *H. sativum.* While variants frequently occur in *Helminthosporium* Spp. (Christensen and Davies (1937)), their production in this instance by amino nitrogen, or in the case of other fungi by nitrite (Steinberg and Thom 1940) seems to offer a clue to their origin. The latter workers considered that the nitrite may have destroyed free amino acid groups in the hereditary mechanism.

The addition of vitamins markedly increased the yield of *H. sativum,* and in some instances, slightly increased that of *C. ramosa.* On the other hand, the growth of *F. culmorum* was depressed. There is probably some relation between this result and the fact that on ordinary potato dextrose agar, *H. sativum* usually makes much slower growth than either *F. culmorum* or *C. ramosa.* It is known that some fungi, at least, are capable of producing vitamins B_1 and C (Lewis 1938, Scheunert et al 1939, Robbins 1939), so that it seems probable, that the degree to which a fungus responds to the addition of vitamins to the media will be dependent on its inherent capacity to produce them itself.

White (1941) has demonstrated that the vegetative development of *Ophiobolus graminis* is dependent on the supply of biotin (Vitamin H) then thiamin (Vitamin B_1) and then a nutritional factor present in wheat straw, wheat roots, peptone and asparagine.

As regards the effects of heavy metal deficiencies, twelve separate experiments were sown as it was found that, even though the dithizone test indicated a low concentration of heavy metals in the purified solutions, there was in many cases sufficient of either of one of the heavy metals to support moderate growth. The particular contaminants were not consistent in each solution.

The results from typical experiments are given in Table 2, and the lowest percentage yield obtained from each deficiency treatments in all experiments with each fungus are set out in Table 3.

TABLE 2.—RESULTS OF TYPICAL EXPERIMENT SHOWING THE EFFECTS OF DEFICIENCIES OF HEAVY METALS ON THE DEVELOPMENT OF ROOT-ROT FUNGI GROWN IN NUTRIENT SOLUTIONS AT 25° C. FOR 14 DAYS.

Treatment.	Fusarium culmorum.		Helminthosporium sativum.		Curvularia ramosa.	
	Dry Weight of Fungus in Grams.	Percentage of Complete Solution.	Dry Weight of Fungus in Grams.	Percentage of Complete Solution.	Dry Weight of Fungus in Grams.	Percentage of Complete Solution.
Complete solution	0·617	100	0·500	100	0·670	100
Manganese deficiency ..	0·450	73	0·410	82	0·435	65
Copper deficiency	0·400	65	0·400	80	0·600	90
Zinc deficiency	0·327	53	0·368	74	0·403	60
Iron deficiency	0·377	61	0·470	94	0·335	50
Difference for Significance	0·034	5·5	0·062	12·4	0·053	7·9

These results indicate that the elements manganese, copper, zinc and iron are each essential to the normal development of some of the common root-rot fungi occurring in Wimmera black soil. In the case of *Phymatotrichum omnivorum* Blank (1941) has demonstrated the existence of important interactions between the elements, particularly for the combinations iron and zinc, and manganese and zinc.

TABLE 3.—LOWEST PERCENTAGE YIELDS OBTAINED FOR EACH HEAVY METAL DEFICIENCY TREATMENT IN ALL EXPERIMENTS.

Treatment.	Fusarium culmorum. Per cent.	Helminthosporium sativum. Per cent.	Curvularia ramosa. Per cent.
Complete solution	100	100	100
Manganese deficiency ..	73	70	60
Copper deficiency	64	80	87
Zinc deficiency	21	48	60
Iron deficiency	24	19	50

Certain features characteristic of deficiencies of these elements were observed. These may be summarised as follows:—

Zinc and Iron Deficiencies:—These treatments led to a very reduced aerial growth, although subsurface growth often occurred. Waksman and Foster (1938) have similarly reported that only a sparse aerial mycelium was formed in their cultures of *Rhizopus nigricans*, which were devoid of iron or zinc, the addition of which profusely stimulated both vegetative development and sporulation.

Manganese Deficiency:—The effects of this treatment were very characteristic, particularly on *H. sativum* and *C. ramosa*. In the early stages growth was very reduced and typical small round colonies occurred either on the surface of the solution or on the bottom of the flask.

Copper Deficiency:—Even though surface growth was often not reduced appreciably, pigmentation during the first few days was practically absent in *F. culmorum*, while in *H. sativum* and *C. ramosa* a pale yellowish-brown to light-greyish colouration was induced. This change in colour is similar to that induced in the spores of *Aspergillus niger* by copper deficiency (Steinberg 1935, Mulder 1938*b*). After approximately ten days, however, the pigmentation of the cultures gradually deepened until it was practically normal.

Discussion.

The experiments have shown that the elements, manganese, copper, iron and zinc, which are known to be essential for the growth of higher plants, are also essential for the normal growth of *F. culmorum*, *H. sativum* and *C. ramosa*. The results of other workers cited earlier have similarly demonstrated that the need for heavy metals for normal growth is a phenomenon manifested by a wide variety of fungi.

Starkey (1938) has found that there can be no doubt that fungi develop vegetatively in the soil in the absence of appreciable amounts of readily decomposed organic matter. Fungus hyphae were found to be abundant even in fallow soil. Garrett (1939) in his review of the literature concluded that certain fungi pathogenic to plants, notably the wilt producing *Fusaria*, are also capable of living as saprophytes on the organic matter in the soil. Further Sadasivan (1939) has shown that *F. culmorum* plays an important part in the early decomposition of normal wheat straw buried in the soil. From their widespread distribution and persistence in the soil and their ready growth on artificial media, it is evident that other root-rot fungi such as *H. sativum* and *C. ramosa* are also capable of living as saprophytes in the soil. In this regard, Sandford (1933) has stated that soil borne pathogens such as *Ophiobolus graminis* and *H. sativum* can utilize soil nutrients for their own vital processes, but what nutrients were essential was not known.

These results therefore indicate that the accumulation in the soil of a large population of root-rot and other saprophytic fungi may effect the growth of the plant indirectly, by competing with the roots of the plant for the soil minerals which are essential to the normal development of both the soil fungi and the plant.

Further investigations relevant to this conclusion are described later in this paper.

Influence of the Soil Flora on the Availability of Soil Minerals.

It has been shown (Starkey 1931, Clark 1940, Lochhead 1940, Timonin 1940*a*) that the presence of plant roots may exercise a profound effect on the soil microflora. Compared with the population in soil distant from the roots, very large accumulations of bacteria and actinomycetes, and to a lesser extent of fungi occur in the rhizosphere.

West and Lochhead (1940) found that these accumulations were associated with the excretion by the roots of stimulative substances such as thiamin, biotin, and amino-nitrogen. Further work by Lochhead et al (1940) and Timonin (1940*b*) has indicated that the rhizosphere of plants susceptible to soil-borne pathogens harboured larger numbers of fungi and bacteria than those of resistant plants. These results suggested the existence of inherent differences between resistant and susceptible varieties, resulting in a more pronounced "rhizosphere effect" in the case of susceptible plants.

Such large populations of micro-organisms would require a greatly increased share of the mineral nutrients in the soil. It is probable that plant roots are unable to compete with the micro-organisms for the soil minerals on an equal basis. In soils, therefore, where a limited available supply of any mineral existed, a deficiency of that mineral in the plant may develop, as a result of the competition of the soil flora, with a consequent injurious effect on growth. It may well be that some of the symptoms associated with soil-borne diseases may be due, in some instances at least, as much to physiological disturbances in the plant arising from this and other causes as to the actual parasitism of the organisms concerned.

The possibility of saprophytic soil fungi and bacteria rendering the soil minerals unavailable to the higher plants has been recognized only comparatively recently. "Grey Speck" disease of oats induced by manganese deficiency (Leeper and Swaby 1940), "Reclamation disease" of oats induced by copper deficiency (Mulder 1938*a*), and "Little Leaf" or "Rosette" disease of fruit trees in America induced by zinc deficiency (Ark 1937, Chandler 1937, Hoagland et al 1937) have been shown to be related to the activities of manganese, copper or zinc fixing soil micro-organisms respectively. It was found that soils in which "little leaf" and related diseases occur can supply an adequate amount of zinc to the plant after they have been sterilized, thus curing the "little leaf" condition. Piper (1938) has also demonstrated that "reclamation disease" of oats in certain areas in South Australia is associated with the lack of available copper in the soil rather than with the total amount present. The availability of the copper to the plant was increased by partial sterilization of the soil, and the plants grown in the autoclaved soil developed normally without any signs of the disease (Waite Institute 1939). In regard to the Robe district in South Australia, it is of interest to note that Riceman and Anderson (1941) have reported that, in the presence of applied copper, the addition of zinc sulphate increased the grain yield of oats. Manganese deficiency may be cured by sterilization with formalin (Gerretson 1935) or by waterlogging the soil (Leeper 1940). In the latter case lack of aeration would considerably curtail the activities of manganese-fixing organisms. Waksman (1931) has discussed the effect of heat in increasing the solubility of soil minerals.

It is recognized that the availability of soil minerals may be affected by factors other than the biological status of the soil. Hibbard (1936, 1940 (*b*)) states that physical, chemical and biological conditions in soil, the nature of the plant and need for specific nutrients, &c., all effect the availability of soil nutrients. Fixation of minerals in the soil may be brought about by anion exchange, molecular adsorption and chemical precipitation. Cation adsorption by bacteria has been demonstrated by McCalla (1940).

It is well established that changes in the soil reaction can appreciably effect the availability of soil minerals (Aleshin and Igritskaia (1938), Chapman et al (1939), Eaton and Wilcox (1939), Hibbard (1940*a*), Leeper (1935), McGeorge (1939), Midgley and Dunklee (1940) and others). It must be remembered, however, that while a change in the soil reaction may effect the availability of minerals, it may concomitantly have and appreciable effect on the biological status of the soil. It is considered that the correlation between these two changes has not been satisfactorily investigated. Leeper (1940) has reported that manganese deficiency disease of oats may be cured either by acidification of the soil below pH 6·5 or by alkalinization with caustic soda raising the pH over 8·5. Waksman (1931) has shown that the optimum reaction for most soil organisms is either around neutral or slightly alkaline, and that bacteria, and to a lesser degree, fungi are limited by increasing acidity. Similarly bacteria, fungi and protozoa are susceptible to increasing alkalinity and most forms are inhibited above pH 9·5. However, few crop plants will grow below pH 3·5 or above pH 9·0.

It may well be that the deleterious effects which sometimes accompany the liming of acid soils may be due in a large degree to the establishment of a more favourable biological environment leading to an increase in the population of soil micro-organisms and a consequent increased demand for the available soil nutrients.

Further reference to the effects of the soil micro-organisms on the availability of the soil minerals is made in the final discussion of the data presented in this paper.

Effects of Soil Sterilization on Mineral Composition of Wheat Plants.

Analyses of wheat plants grown in the Wimmera on black ground (Millikan 1940) have shown that even plants receiving no zinc sulphate have a relatively high zinc content. This indicates that the responsiveness of plants grown in Wimmera black soil to applications of zinc sulphate is not related to a complete lack of zinc in the rhizosphere, but rather to a slightly insufficient supply in an available form. This insufficiency was made good by treating the plants with zinc sulphate, and the small increase in the zinc concentration which resulted from this treatment was accompanied by a marked improvement in the general growth of the plants.

If, as suggested above, the deficiency of zinc available to the plant in the soil be due to competition between the plant and the soil flora for the soil zinc, it should also be possible to make up this insufficiency by sterilizing the soil to eliminate this competition, providing an adequate total amount of zinc existed in the soil.

Experiments have been conducted at the Plant Research Laboratory, Burnley, during the three seasons 1938-40 inclusive, to determine, in the first instance, whether the availability to the plant of the zinc and other minerals in Wimmera black and red soils was affected by soil sterilization.

Method.

In 1938 and 1939 the comparative effects of the application of zinc sulphate in conjunction with superphosphate to sterilized and unsterilized Wimmera black fallow soil from Nhill and Salisbury were studied.

In the first (1938) season metal containers six inches in diameter and six inches deep were used to hold the soil. The inside of each container was coated with a thin layer of high grade paraffin wax before use and the superphosphate and zinc sulphate were mixed with the top two inches of soil only. The treatments studied in both unsterilized and sterilized soil were:—Superphosphate 1½ cwt. per acre, and Superphosphate 1½ cwt. + Zinc sulphate 30 lb. per acre, respectively. The soils were sterilized by autoclaving at two atmospheres pressure for two hours. The soils were air dry at the time of sterilization. Seven plants of Free Gallipoli wheat were grown to maturity in each pot, while the moisture content of the soil was kept constant during the experiment at 50 per cent. of its waterholding capacity by the addition of distilled water every second day. Each treatment was replicated four times.

During 1939 Nhill black fallow soil only was used in paraffined wooden boxes two feet square by six inches deep. The treatments studied were the same as in the previous year. The zinc sulphate and/or superphosphate were mixed with the top two inches of soil in each box. Free Gallipoli wheat was again used, the grains being spaced one inch apart each way. One composite sample of plants was taken at four, six and nine weeks after germination from the three replicates of each treatment, and were submitted to the Agricultural Research Chemist for ash analyses.

A study of the comparative effects of soil sterilization, of Nhill black and red fallow soils respectively, on the mineral composition of wheat plants subsequently grown therein was made in 1940.

Paraffined wooden boxes were again used, but were two inches deeper than those used in the previous season. No superphosphate or zinc sulphate was applied to the soils, and the sterilization treatment of air dry soil was at $\frac{1}{2}$ atmosphere for $\frac{3}{4}$ hour, immediately prior to sowing. Each treatment was replicated three times. Composite samples of plants from the three replicates of each treatment were obtained at four and eight weeks after germination and were submitted to the Agricultural Research Chemist for ash analyses.

In the field at Nhill, wheat plots were sown without fertilizers on the same red and black fallow areas respectively from which the soils for the 1940 experiments at Burnley were obtained. Ash analyses were made by the Agricultural Research Chemist on plant samples obtained from these plots at six weeks after germination to determine whether the mineral compositions of the plants grown under field conditions differed to any marked degree from those of the plants grown in the same soils at Burnley.

Results.

1938.—The growth obtained in the sterilized soil was very much superior to that which occurred in unsterilized soil, both as regards height and depth of colour. The plants in the unsterilized soil developed a marked yellowing of the tips of the leaves such as occurs commonly in the field at Nhill.

In unsterilized soil, an improvement in growth due to the application of zinc sulphate was first noticeable when the plants were in the third leaf stage. Later the zinc treated plants became somewhat taller and showed better tillering.

In the sterilized soil the zinc treatment had no apparent effect on growth up to the heading stage. At this stage, however, it was observed that soil sterilization exercised a profound influence on the response of the wheat to zinc applications. Whereas in the unsterilized soil the effect of the zinc sulphate application was similar to that resulting under field conditions (i.e., to induce the earlier appearance of the ears), the zinc treatment in the sterilized soil consistently retarded the date of appearance of the ears by one week. (Plate X., fig. 5.)

1939.—The effect of soil sterilization on growth was first noticeable three weeks after germinations. At this stage the plants growing in unsterilized soil showed poorer growth, were paler green in colour, and in many instances the tips of the leaves were beginning to brown. Later the development of the plants growing in unsterilized soil became very inferior to that of the plants in sterilized soil.

As regards the effects of the zinc sulphate applications, little difference in growth at nine weeks after germination was induced in the plants growing in unsterilized soil. It was observed, however, in the case of the sterilized soil series, that the average weight per plant in samples obtained at six and nine weeks after germination was reduced by the addition of zinc sulphate. This confirms the observation made in the previous season that the application of zinc sulphate to sterilized Wimmera black soil had a harmful effect on the growth of wheat.

The analyses of the plants shown in Table 4 indicate that the sterilization of the soil had a profound effect on the mineral composition of the plants. Their silica (SiO_2), and lime (CaO) concentrations were reduced, while increases in their phosphoric acid (P_2O_5), potash (K_2O), zinc (Zn), manganese (Mn), and nitrogen (N) concentrations occurred. Not only was the percentage of these latter constituents increased but the total amount of them absorbed would be very much greater than in the unsterilized soil owing to the great improvement in growth promoted by soil sterilization.

TABLE 4.—SHOWING THE EFFECT OF STEAM STERILIZATION OF NHILL BLACK FALLOW SOIL ON THE MINERAL COMPOSITION, AT FOUR, SIX AND NINE WEEKS AFTER GERMINATION, OF FREE GALLIPOLI WHEAT GROWN THEREIN AT BURNLEY DURING 1939. RESULTS OF ANALYSES AS PER CENT. ON DRY BASIS.

—	Unsterilized.		Sterilized.	
	Superphosphate 1½ cwt. per Acre.	Superphosphate 1½ cwt. + Zinc Sulphate 30 lb. per Acre.	Superphosphate 1½ cwt. per Acre.	Superphosphate 1½ cwt. + Zinc Sulphate 30 lb. per Acre.
(a) Analyses Four Weeks after Germination.				
Crude Ash	14·45	14·63	17·41	17·86
Silica Free Ash	9·88	9·99	13·08	12·93
Silica (SiO_2)	4·57	4·64	4·33	4·93
Phosphoric Acid (P_2O_5)	1·57	1·74	2·53	2·60
Lime (CaO)	1·07	1·11	0·96	0·95
Potash (K_2O)	3·77	3·70	5·24	5·51
Zinc (Zn) parts per million	34	39	54	78
(b) Analyses Six Weeks after Germination.				
Crude Ash	13·67	14·48	17·37	16·79
Silica Free Ash	8·49	8·27	14·07	13·59
Silica (SiO_2)	5·18	6·21	3·30	3·20
Lime (CaO)	1·16	1·17	0·94	0·92
Phosphoric Acid (P_2O_5)	1·17	1·22	3·60	3·42
Potash (K_2O)	3·10	2·61	5·93	5·95
Zinc (Zn) parts per million	31	35	57	74
(c) Analyses Nine Weeks after Germination.				
Nitrogen	1·20	1·21	2·42	2·50
Crude Ash	19·41	16·90	14·86	14·02
Silica Free Ash	7·00	6·49	9·20	9·67
Silica (SiO_2)	12·41	10·41	5·66	4·35
Lime (CaO)	1·10	1·15	0·81	0·79
Phosphoric Acid (P_2O_5)	0·68	0·75	2·22	2·26
Potash (K_2O)	2·32	2·25	4·22	4·45
Zinc (Zn) parts per million	32	33	56	66
Manganese (Mn) parts per million ..	114	107	143	183

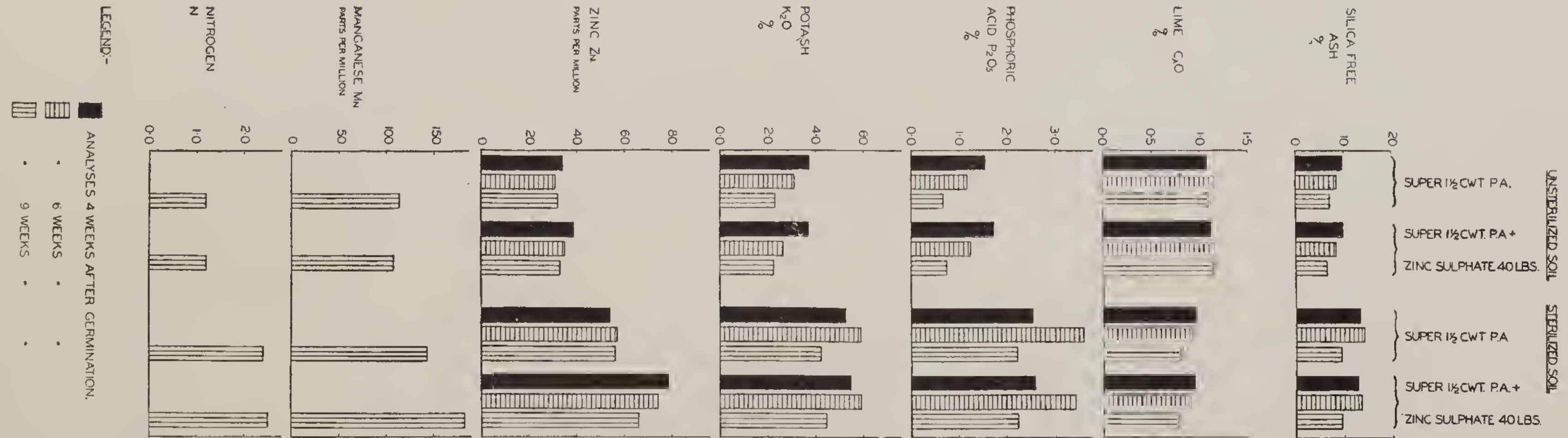


FIG. 1.—Results of analyses given in Table 4, showing the effect of steam sterilization of Nhill black fallow soil on the mineral composition at four, six and nine weeks after germination, of Free Gallipoli Wheat grown therein at Burnley during 1939. Analyses on dry basis.

The fact that the concentration of zinc present in the plants grown in sterilized soil treated with superphosphate only, was considerably higher than in the unsterilized soil series plants treated with superphosphate plus zinc sulphate, indicates that when sterilized, the Wimmera black soil is capable of supplying an adequate amount of zinc in a form readily available to the plant.

From these figures it also appears probable that the application of zinc sulphate to sterilized soil may even increase the amount of this element available to the plant above the optimum point and thus induce some deleterious effect on the growth of the plant, such as retardation in the date of heading noted in 1939 or decreased weight per plant referred to above. The analyses show that such plants contained the highest concentration of zinc. Although plants require minute quantities of elements such as zinc, manganese and copper for normal growth Arnon and Stout (1939) have stated that most plants are also injured by very small increases in the concentrations of such elements in the plant.

The zinc concentration reaches a maximum early in the development of the plant and subsequently falls to a very low figure after heading. It is probable that the total amount of zinc in the plant does not decrease after the maximum concentration has been passed but that the percentage decreases due to " dilution " with dry matter.

1940.—Similar improvements in growth to those described above for the previous two seasons resulted from the steam sterilization of both red and black fallow soils from Nhill. The unsterilized soil plants, besides being not as tall as the plants grown in sterilized soil, were pale green in colour and showed marked yellowing of the tips of the leaves; these latter symptoms were cured by soil sterilization. Yellowing of the tips of the leaves also occurred in the case of the plants under field conditions at Nhill.

A comparison of the analyses of these field grown plants at six weeks after germination, with those of plants grown in the same soils in boxes at Burnley at four and eight weeks after germination, show very close agreement after allowance is made for the variations in the percentages of the constituents which occur with age (Table 5).

In the black soil, the changes in the mineral constituents of the plants induced by sterilization were similar to those recorded in 1939, i.e., the percentages of phosphoric acid (P_2O_5), potash (K_2O), zinc and manganese were increased. In the 1940 series the percentages of lime (CaO) and copper were also increased in the plants grown on sterilized soil. Sterilization had no effect on the percentage of magnesia (MgO) in either season.

In the plants grown on red soil, sterilization induced similar changes in the percentages of mineral constituents to those which occurred on black soil, i.e., the percentages of lime (CaO), phosphoric acid (P_2O_5), potash (K_2O), magnesia (MgO), zinc, manganese and copper were increased. The relative increase in the percentage of manganese was much greater in red than in black soil, while only a slight increase in the precentage of zinc was recorded for the former soil at eight weeks after germination.

An interesting effect of soil sterilization common to both soils was a decrease in the percentage of iron (Fe) in the plants. The reason for this change is at present unknown. It may be associated with some antagonistic effect in the plant resulting from the other profound changes induced in mineral composition by soil sterilization.

In view of the much superior growth of the sterilized soil plants compared with those grown in unsterilized soil, however, it is possible that the total amount of iron absorbed was not decreased by sterilization.

One further point to be noted from Table 5, is that differences in the normal percentages of mineral constituents occurred between the plants growing on Wimmera black and red soils respectively. The red soil plants contained the highest percentages of phosphoric acid (P_2O_5), zinc and copper (the latter at eight weeks after germination) and the lowest percentages of lime (CaO), potash (K_2O), manganese and iron. The percentages of magnesia (MgO) and nitrogen (N) recorded for the two types of soil were similar.

These differences in the normal composition of the plants grown on the two types of soil are probably closely connected with, firstly, the lack of response in wheat to applications of zinc sulphate in the field; secondly, the relatively smaller response on red than on black fallow soil from the application of such mineral mixtures as have been applied to date. The latter effect is described in detail later in this paper. (Tables 8, 10, and 11.)

These experiments have shown that certain plant nutrients become more readily available to the plant as a result of the sterilization of Wimmera red and black soils leading to improved growth and colour in the plants. This result is similar to that reported by Piper (1938), and cited above, in relation to copper deficiency disease of oats in South Australia.

It was recognized that the availability of soil minerals can be appreciably affected by changes in the soil pH. The reactions of steam sterilized and unsterilized black and red fallow soils from Nhill have been determined, to discover whether the marked increases in mineral availability recorded above as a result of steam sterilization of these soils could be attributed, in part at least, to possible changes in pH induced by the heat treatment.

From the results below it will be seen that steam sterilization had no effect on the reaction of the soil:—

Black fallow soil—unsterilized	pH. 8·5
Black fallow soil—steam sterilized	pH. 8·5
Red fallow soil—unsterilized	pH. 6·2
Red fallow soil—steam sterilized	pH. 6·3

TABLE 5.—ANALYSES, ON DRY BASIS, OF FREE GALLIPOLI WHEAT PLANTS GROWN ON WIMMERA RED AND BLACK FALLOW SOILS RESPECTIVELY AT NHILL AND BURNLEY DURING 1940, SHOWING NORMAL DIFFERENCES IN MINERAL COMPOSITION OF THE PLANTS, AND THE COMPARATIVE EFFECTS OF STERILIZATION OF EACH SOIL ON THE AVAILABILITY OF MINERALS TO THE PLANT.

—	Red Fallow Soil.			Black Fallow Soil.		
	Field Grown Plants (Nhill).	Unsterilized Soil (Burnley).	Sterilized Soil (Burnley).	Field Grown Plants (Nhill).	Unsterilized Soil (Burnley).	Sterilized Soil (Burnley).
(a) Analyses Four Weeks after Germination.						
Lime (CaO) per cent.	..	0·43	0·65		0·61	0·78
Magnesia (MgO) per cent. ..	..	0·49	0·70	..	0·48	0·49
Potash (K_2O) per cent.	..	4·57	5·50	..	5·23	5·72
Phosphoric acid (P_2O_5) per cent. ..	..	1·60	2·51	..	1·05	1·93
Zinc (Zn) parts per million ..	..	54	52	..	30	45
Copper (Cu) parts per million ..	..	17	21	..	16	15
Manganese (Mn) parts per million ..	..	38	154	..	90	133
Iron (Fe) parts per million ..	..	21	24	..	84	59
(b) Analyses Six Weeks after Germination.						
Magnesia (MgO) per cent. ..	0·50	..	..	0·49	..	..
Phosphoric Acid (P_2O_5) per cent. ..	0·76	..	..	0·71	..	..
Nitrogen (N) per cent.	5·77	..	..	5·55	..	..
Zinc (Zn) parts per million ..	28	..	..	21	..	..
Copper (Cu) parts per million ..	18	..	..	18	..	..
Manganese (Mn) parts per million ..	46	..	..	70	..	..
Iron (Fe) parts per million ..	68	..	..	85	..	..
(c) Analyses Eight Weeks after Germination.						
Lime (CaO) per cent.	..	0·43	0·56	..	0·82	0·96
Magnesia (MgO) per cent. ..	..	0·39	0·37	..	0·35	0·35
Potash (K_2O) per cent.	..	3·58	5·06	..	4·43	5·94
Phosphoric Acid (P_2O_5) per cent. ..	..	0·86	1·65	..	0·63	1·85
Zinc (Zn) parts per million ..	..	30	36	..	27	47
Copper (Cu) parts per million ..	..	24	25	..	5	8
Manganese (Mn) parts per million ..	..	50	251	..	49	167
Iron (Fe) parts per million ..	..	45	25	..	35	27

Further, as shown in Table 8, soil sterilization with 2 per cent. formalin produced a similar improvement in growth to that resulting from steam sterilization.

EFFECTS OF RE-INOCULATION OF STERILIZED SOIL WITH SOIL INHABITING ORGANISMS.

Preliminary investigations designed to be complementary to the soil sterilization studies reported above have been conducted at Burnley during 1939 and 1940. The object of this work was to

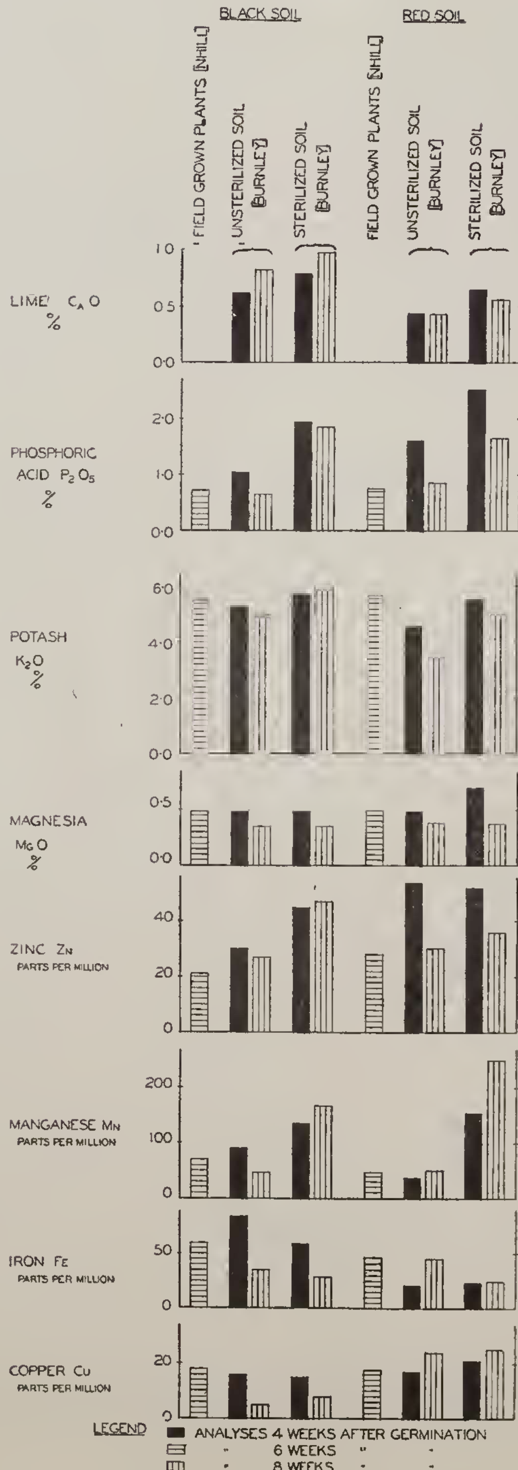


Fig. 2.—Results of analyses, given in Table 5, of Free Gallipoli wheat plants grown on Wimmera red and black fallow soils respectively at Nhill and Burnley during 1940, showing normal differences in the mineral composition of the plants, and the comparative effects of sterilization of each soil on the availability of minerals to the plant. Analyses on dry basis.

determine whether the activities of soil-inhabiting bacteria and fungi in the rhizosphere in Wimmera soil affect the growth of the plant indirectly by using the soil nutrients for their own vital processes, thus reducing the amount available to the plant.

The results of nutritional studies on the heavy metal requirements of soil-inhabiting root-rot fungi have been described above. In addition to this work experiments were made on the mineral relationship of wheat plants grown in sterilized Wimmera black fallow soil re-inoculated with some of the normally occurring soil fungi and bacteria.

Method.

In 1939 portions of air dry black fallow soil were steam sterilized at two atmospheres pressure for two hours, and then inoculated with a mixed suspension of soil bacteria in water two months prior to sowing with Free Gallipoli wheat. After inoculation the moisture content of the soil was kept constant at 50 per cent. of its water holding capacity. In the meantime a further portion of the original soil was kept air dry and was sterilized immediately prior to sowing. Paraffined containers 6 inches in diameter and 6 inches deep were used to hold the soil. At sowing time the top two inches of soil in each container was treated with either superphosphate $1\frac{1}{2}$ cwt. per acre or superphosphate $1\frac{1}{2}$ cwt. + Zinc sulphate 30 lb. per acre respectively. Each treatment was replicated four times and seven grains were sown in each pot. The moisture content of the soil was kept constant by the addition of distilled water every second day.

In 1940 paraffined wooden boxes 2 feet square by 8 inches deep were used to hold the soil which was sterilized two months before sowing by autoclaving at half an atmosphere pressure for $\frac{3}{4}$ hour. Separate portions of this sterilized soil were immediately re-inoculated with a suspension in water of one of the following mixtures of organisms:—Root-rot fungi (*Curvularia ramosa, Helminthosporium sativum, Fusarium culmorum*), Saprophytic fungi (*Rhizopus sp.* and *Pennicillium spp.*), or a mixture of soil bacteria. To a further portion of sterilized soil 1 per cent. of unsterilized soil was added.

The control treatments of sterilized soil only and unsterilized soil were set up at this time and were maintained under comparable conditions to the inoculated series.

In the meantime a further portion of unsterilized soil was kept air dry and was sterilized immediately prior to sowing. Each treatment was replicated three times. No fertilizer was applied in this experiment, and approximately 500 grains of wheat were sown in each box.

Composite samples of plants were obtained from the three replicates of each treatment at both four and eight weeks after germination and were submitted to the Agricultural Research Chemist for ash analyses.

Results.

1939.—In the uninoculated sterilized soil, it was found that the application of zinc sulphate retarded growth, in contrast to the stimulation of growth promoted by this treatment in the unsterilized soil. These effects were in accord with the results of other experiments with fallow soil reported above. The retardation of growth in sterilized soil was first noticeable in the fourth leaf stage, six weeks after germination.

On the other hand, the zinc sulphate application to sterilized soil re-inoculated with bacteria definitely stimulated growth. This stimulation was first noticeable approximately eight weeks after germination.

From this preliminary experiment it was evident that the zinc responsiveness of Wimmera black fallow soil which is destroyed by steam sterilization can be re-established by inoculating such sterilized soil with soil bacteria.

1940.—The big improvement in growth due to soil sterilization which occurred in this experiment was similar to that described above for other experiments, where a similar treatment was given. The growth in the soil sterilized two months prior to sowing was slightly superior to that in the soil sterilized immediately prior to sowing.

At the time of first sampling four weeks after germination, no obvious differences in growth between the sterilized only soils and the sterilized soils re-inoculated with soil organisms was discernable.

At eight weeks after germination, however, when the second samples were taken, the plants in the inoculated sterilized soils appeared slightly paler green in colour than the sterilized soil controls. The tips of the leaves of the plants in the sterilized soil inoculated with root-rot fungi were slightly yellowed.

The results of the analyses made on these samples are shown in Table 6. Several points of some significance may be noted from a study of these figures. In the first instance a gap of two months between sterilization and sowing evidently had a beneficial effect on the availability of potash (K_2O), magnesia (MgO), and manganese, to the plant. On the other hand, the lime (CaO) and phosphoric acid concentrations were higher in the plants grown in soil sterilized immediately prior to sowing. It is known that after soil sterilization, time is sometimes required before the full benefit of the treatment on subsequent plant growth is achieved.

A time factor doubtless operates in relation to the autolysis of soil organisms subsequent to soil sterilization, and the concomitant release of plant nutrients.

TABLE 6.—PRELIMINARY EXPERIMENT WITH WIMMERA BLACK FALLOW SOIL CONDUCTED AT BURNLEY DURING 1940, SHOWING COMPARATIVE EFFECTS OF STERILIZATION TWO MONTHS PRIOR TO SOWING, IMMEDIATE RE-INOCULATION OF SUCH STERILIZED SOIL WITH SOIL FUNGI OR BACTERIA, AND STERILIZATION JUST PRIOR TO SOWING RESPECTIVELY, ON THE MINERAL COMPOSITION OF FREE GALLIPOLI WHEAT PLANTS GROWN THEREIN. ANALYSES ON DRY BASIS.

—	Unsterilized.	Sterilized (April).	Sterilized (June).	Sterilized (April) + Root Rot Fungi.	Sterilized (April) + Saprophytic Fungi.	Sterilized (April) + Soil Bacteria.	Sterilized (April) + 1 per cent. Unsterilized.
(a) Analyses Four Weeks after Germination.							
Lime (CaO) per cent.	0·61	0·75	0·78	0·89	0·85	0·76	0·89
Mangesia (MgO) per cent. ..	0·48	0·48	0·49	0·51	0·52	0·56	0·52
Potash (K_2O) per cent.	5·23	5·50	5·72	5·61	5·68	6·11	5·50
Phosphoric acid (P_2O_5) per cent. ..	1·05	1·75	1·93	1·55	1·77	1·52	1·45
Zinc (Zn) parts per million ..	30	41	45	47	46	49	46
Copper (Cu) parts per million ..	16	20	15	24	34	30	15
Iron (Fe) parts per million ..	84	55	59	71	50	48	45
Manganese (Mn) parts per million	90	138	133	123	120	117	130
(b) Analyses Eight Weeks after Germination.							
Lime (CaO) per cent.	0·82	0·81	0·96	0·94	0·94	0·93	0·90
Magnesia (MgO) per cent. ..	0·35	0·47	0·35	0·34	0·35	0·38	0·46
Potash (K_2O) per cent. ..	4·43	6·93	5·94	6·17	6·40	6·69	6·57
Phosphoric Acid (P_2O_5) per cent...	0·63	1·48	1·85	1·61	1·53	1·46	1·11
Zinc (Zn) parts per million ..	27	49	47	41	50	44	36
Copper (Cu) parts per million ..	5	7	8	9	14	9	9
Iron (Fe) parts per million ..	35	31	29	29	29	26	31
Manganese (Mn) parts per million	49	203	167	140	149	161	151

A second point of importance to be noted from the results is that the general nutritional level of the plants in the sterilized re-inoculated soils even four months after inoculation is still very much higher than that of the unsterilized soil plants. It is evident, therefore, that to obtain the full effects of re-inoculation of sterilized soil, analyses should be made later than eight weeks after germination. Probably more reliable information would be obtained by making analyses on the "residual effect" in stubble sown plants in the season after inoculation. Fungi often grow only moderately well on sterilized soil without the addition of stimulating substances. Such substances are secreted by plant roots. References to this "rhizosphere effect" have been cited at the beginning of this section. It seems probable, therefore, that the increase in the organisms inoculated into sterilized soil would be greater after the plants had become established than before.

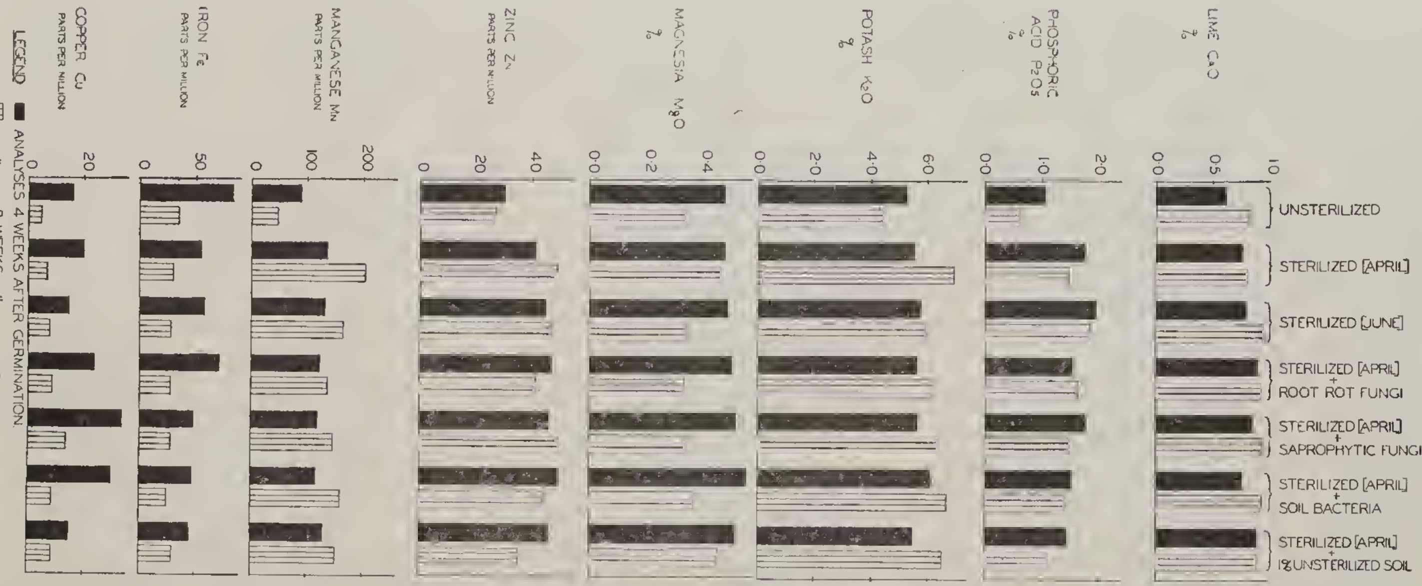


FIG. 3.—Results of analyses, given in Table 6, of a preliminary experiment with Wimmera black fallow soil conducted at Burnley during 1940, showing comparative effects of sterilization two months prior to sowing, immediate re-inoculation of such sterilized soil with soil fungi or bacteria, and sterilization just prior to sowing, respectively, on the mineral composition at four and eight weeks after germination of Free Gallipoli wheat plants grown therein. Analyses on dry basis.

This latter is in accord with the analyses of the plants. Whereas the inoculations of sterilized soil had little effect on the percentage composition of the plants at four weeks after germination, the analyses four weeks later show some marked changes. Compared with the control plants in Sterilized (April) Soil, the analyses eight weeks after germination showed lower percentages of phosphoric acid (P_2O_5), potash (K_2O), magnesia (MgO), zinc and manganese in some or all of the inoculation treatments.

The results of this preliminary experiment have shown that the presence of soil inhabiting organisms in the rhizosphere may decrease the availability of certain mineral nutrients to the plant.

The variable results reported from inoculation tests using sterilized soil may well be due to the fact that the effects of the competition between the plant and the organisms for the soil minerals was not apparent, owing to the marked increase in the amount of available soil minerals due to the sterilization treatment. The time factor is obviously very important in all such experiments. This may be the explanation of the results of Sandford (1941) who found that *Rhizoctonia solani* was not virulent in sterilized soil.

Mineral Treatment in Relation to the Apparent Severity of Root-rot Disease.

The literature on the effects of manuring and other treatments on the development of soil-borne diseases in plants has been reviewed by Garrett (1939).

Deficiencies of available plant nutrients in the soil may be induced by a variety of causes; some references have been cited earlier. Where such deficiencies exist, the resulting symptoms in the plant such as restricted growth and reduced yield, would, if associated with the occurrence of root-rot lesions in the plants, be liable to be wrongly attributed to the direct effects of root-rot organisms. The apparent severity of the root-rot disease would therefore appear to be greater than its actual severity. Alleviation of the soil deficiency by suitable treatment may therefore decrease the apparent severity of the disease without actually decreasing the number of lesions on the roots of the affected plants. This is probably the explanation of the results of Russell and Sallans (1940), who found that although the applications of phosphatic fertilizers frequently increased the yield of wheat, there was usually, if anything, a slight increase in the common root-rot disease rating of the fertilized plants. In view of the increased yields produced by the fertilized plants, however, it is obvious that the apparent severity of the disease must have been reduced.

Other instances have been recorded where the occurrence of soil mineral deficiencies had appreciable effects on the apparent severities of disease in susceptible plants grown in those soils

(Samuel 1934, Miles 1936, Millikan 1938, Walker and Musbach 1939, Vanterpool 1940). The application of the appropriate mineral not only cured the deficiency, but also reduced the apparent severity of the disease. This latter beneficial effect is evidently bound up more with the curing of a physiological disorder, resulting in an increase in the vigor and yield of the plant, than with any increase in the plant's resistance to fungal attack.

In a study of the root-rot problem, it is therefore of paramount importance to distinguish between symptoms caused by physiological disorders in the plant induced by external factors and those caused by the direct effects of the root-rot organisms.

The results recorded earlier in this paper have indicated that the fungi and bacteria inhabiting Wimmera black soil may exercise an indirect, harmful effect on plant growth by competing with the plant for the soil nutrients. It would follow, therefore, that a mineral treatment would help to overcome the indirect effects of these organisms on plant growth by supplying additional nutrients in a form immediately available to the plant, thus producing an improvement in growth at least approaching that resulting from steam sterilization.

Where other environmental factors such as soil moisture were optimum for growth, the presence of soil inhabiting organisms which may become plant pathogens would tend to prevent such a treatment from inducing an improvement in growth completely comparable with the effect of sterilization. Further, to obtain the maximum response in growth the ingredients of the treatment applied would necessarily need to be of a proper kind and proportion to meet the particular needs of the soil concerned. Such a treatment could only be developed after much research.

Under these conditions, the relative growths obtained in the sterilized soil, and in unsterilized soil supplied with additional nutrients, would give an indication as to the real effects on plant growth of the root-rot organisms present in that soil. Such an experiment, it seems to the writer, is the only means by which the actual pathogenicity of root-rot organisms can be evaluated.

To determine the effects of mineral treatment on the development of cereals grown on Wimmera soil, experiments have been conducted in the greenhouse at the Plant Research Laboratory, Burnley, in 1939 and 1940, and in the field on the farm of Mr. C. P. Dahlenburg, Nhill, during the seasons 1938 to 1940 inclusive. The effects of such treatments on the abundance of root-rot lesions on the plant was also determined in the field experiments.

Greenhouse Experiments, 1939-40.

Nhill black wheat stubble soil was selected for the first experiment which was conducted during 1939. The growth of wheat and oats on such soil is usually very poor. The following year the experiments were extended to include Nhill black and red fallow soils in addition to the black stubble soil.

Method.

Each treatment was replicated four times and all fertilizers and minerals were applied to the top two inches of soil only in each container, which measured six inches in diameter by six inches deep. Each was coated with a thin layer of high grade paraffin before use. With the exception of the first experiment in 1939, the soils at the time of sterilization were air dry, and the treatment was for three-quarters of an hour at one-half atmosphere pressure.

Before sowing, the moisture content of the soil was adjusted to 50 per cent. of its moisture holding capacity, and was kept constant at this figure during the experiment by the addition of distilled water thrice weekly. Seven grains of Free Gallipoli wheat were sown in each pot.

At the termination of the 1940 experiment, the dry weights of the plants were obtained.

The treatments studied in each season were as follow :—

1939.—A dressing of either superphosphate 1½ cwt. per acre, or superphosphate 1½ cwt. + zinc sulphate 40 lb. per acre was applied to unsterilized soil, sterilized soil (half atmosphere for half hour) and sterilized soil (two atmospheres for two hours) respectively. A further treatment consisting of unsterilized soil + mineral mixture (see Table 7 for composition) was included.

194C.—The treatments applied to each type of soil in 1940 are shown in Tables 7 and 8.

Table 7.—Greenhouse Wheat Experiments, Burnley, 1939-40, Showing the Compositions, in Pounds per Acre, of the Mineral Mixtures Applied to the Various Types of Nhill Soil (i.e., Black Fallow, Black Wheat Stubble and Red Fallow) Used.

Constituent.	1939.	1940 Mixtures.							
		1.	2.	3.	4.	5.	6.	7.	8.
Superphosphate	168	112	168	168	168	56	56	168	168
Sulphate of ammonia ..	112	28	112	112	112	112	112	112	112
Zinc sulphate	40	20	20	20	20	..	..	20	..
Manganese sulphate ..	40	15	15	30	30	30	30	15	..
Sulphate of potash ..	..	25	25	25	25	25	25	25	25
Magnesium sulphate ..	30	..	..	20	30	30	..	20	20
Copper sulphate	4	..	..	3	4	4	..	5	..
Iron sulphate	10	..	..	15	15	15	15	15	..
Cobalt chloride	2	..	..	2	2	2	..	4	4
Ammonium molybdate ..	2	..	..	2	2	2	..	4	4
Nickel sulphate	1	..	..	2	2	2	..	4	4
Borax	..	..	..	2	2	2	..	..	..

Results.

Marked differences in growth were induced by certain of the treatments (Plate X., fig. 6; Plate XI., fig. 7). As seen in Table 8 the greatest increases were promoted by soil sterilization with either steam or formalin. The mineral mixtures also induced appreciable increases in growth, particularly in the black stubble soil in both 1939 and 1940. In this soil the appearance of the mineral treated plants up to the heading stage was only very slightly inferior to that of the sterilized soil plants. Growth on stubble is normally much inferior to that on fallow.

One point of particular interest revealed in Table 8 is that whereas the addition of the elements magnesium, copper, cobalt, molybdenum, nickel and boron to the mixtures applied to black fallow soil significantly depressed the yield, their inclusion in the black stubble soil mixtures caused a very appreciable increase in the weight of dry matter produced. Evidently the process of fallowing Nhill black soil increases the availability of some, at least, of the elements listed above. In this regard it is of interest to note that in Western Australia the growth of " rosetted " pines suffering from zinc deficiency (Kessell and Stoate 1936) has been found to be stimulated by injections of manganese, iron, cobalt, nickel, molybdenum and boron in addition to zinc (Hearman 1938). Similarly Riceman and Anderson (1941) have reported that in the Robe area in South Australia the addition of zinc sulphate in the presence of applied copper, increased the grain yield of oats considerably.

Although the colour of the plants grown in the red soil was lighter than that of the black fallow soil plants, the dry weight per plant of the former was greatest. In the field, on the other hand, the yield on black ground is usually superior to that on red ground. This is due largely to the fact that the moisture holding capacity of red ground is not as great as that of the black, and plants in the former type of soil are consequently liable to suffer most from a lack of moisture. This limiting factor was eliminated in the greenhouse experiments.

The occurrence of " dead-heads " which is a common feature of red-ground crops in the Wimmera district (Plate XII., fig. 11) is also considered to be closely correlated with the moisture relations of the plants. They occur most commonly under dry conditions. Further reference is made to this aspect in the discussion later. It will suffice to state here that while no " dead-heads " developed in the plants in the greenhouse experiment, they were very prevalent in plants grown in the same season and soil under very dry field conditions.

No accurate quantitative determinations of the severity of root-rot lesions on the roots of the plants in the greenhouse experiments were possible. It was observed, however, that root development was much superior in the plants receiving the mineral mixtures, which resulted in decreases in the apparent severity of the lesions.

The significance of these results is discussed after a description of complementary field experiments.

TABLE 8.—RESULTS OF GREENHOUSE EXPERIMENTS, 1940, SHOWING THE COMPARATIVE EFFECTS OF STERILIZATION AND MINERAL TREATMENT OF NHILL SOILS ON THE DRY WEIGHTS OF FREE GALLIPOLI WHEAT PLANTS GROWN THEREIN.

Treatment.	Black Fallow.		Black Wheat Stubble.		Red Fallow.	
	Dry Weight per Plant Grams.	Percentage Increase.	Dry Weight per Plant Grams.	Percentage Increase.	Dry Weight per Plant Grams.	Percentage Increase.
Unsterilized	0·328	..	0·119	..	0·823	..
Unsterilized + Superphosphate 1 cwt. per acre	0·437	33	..	..	..	..
Unsterilized + Superphosphate 1½ cwt. per acre	..	..	0·114	4*	0·834	1
Unsterilized + Mineral Mixture No. 1	0·572	74	..	..	..	..
Unsterilized + Mineral Mixture No. 2	0·606	85	..	..	..	..
Unsterilized + Mineral Mixture No. 3	0·485	48	..	..	..	..
Unsterilized + Mineral Mixture No. 4	..	..	0·353	198	..	..
Unsterilized + Mineral Mixture No. 5	..	..	0·325	173	..	..
Unsterilized + Mineral Mixture No. 6	..	..	0·231	94	..	..
Unsterilized + Mineral Mixture No. 7	..	..	..	..	1·056	28
Unsterilized + Mineral Mixture No. 8	..	..	..	..	1·073	30
Sterilized (steam)	0·801	144	0·608	411	1·544	88
Sterilized (formalin)	0·845	150	..	..	..	..
Sterilized (steam) + Mineral Mixture No. 3	1·014	209	..	..	..	..
Difference for significance ..	0·117	36	0·074	62	0·211	26

* Decrease.

NOTE.—For compositions of Mineral Mixtures see Table 7.

FIELD EXPERIMENTS, 1938-40.

On the farm of Mr. C. P. Dahlenburg, Nhill, the effects of mineral treatments on the growth and disease reaction of wheat on black and red fallow ground, and of oats on black wheat stubble soil have been studied during 1938-40 inclusive. The experiments were intended to be complementary to the greenhouse work described above.

Method.

The various minerals were mixed with superphosphate in the desired proportions and applied with the drill at seeding time. The compositions of the mineral mixtures used in each season are shown in Table 9, and tests in which they were included are indicated in Tables 10, 11, and 12.

All plots were 0·0322 acres in area, and each treatment was replicated three or four times in randomized blocks.

During the season, random samples of approximately 60 plants were obtained from each plot and were examined for the prevalence of root-rot lesions. For comparison purposes, two samples were taken from the black fallow experiments, the first just prior to heading and the other very shortly before harvest when the roots were becoming senile.

The examination took into account the number of lesions in relation to the root development and general vigour of each plant, which was then allotted points according to the following schedule:—Very severe, 5 points; severe, 4 points; moderate, 3 points; light, 2 points; very light, 1 point; and no lesions, nil. The root-rot index of the sample was then obtained as follows:—

$$\text{Root-rot Index} = \frac{\text{Total points scored}}{(\text{Total number of plants in sample}) \times 5} \times \frac{100}{1.}$$

The yields of either grain or hay were obtained at the end of each season.

Results.

In each season, particularly the early portion, certain of the mineral mixtures tested produced very much superior growth in both wheat and oats to that induced by zinc sulphate alone (Plate XI., fig. 8; Plate XII., fig. 12). The greatest increases occurred in oats on stubble. Unfavorable climatic conditions in the latter part of each season, however, adversely affected the growth of the mineral treated plants in relation to that of the other treatments.

During 1938 and 1940 drought conditions were experienced, and the plants which were most forward early in the season suffered most later from the continued dry weather. In 1938 practically all the oats on stubble died before reaching maturity, while in 1940 they were too short to cut. In 1939 a series of very severe frosts occurred just prior to and during the heading of the mineral treated plants (which were the first to mature) while the later plants not receiving the mixtures escaped serious injury.

For these reasons, the differences in the final yields obtained from the various treatments were not in accordance with the early and mid-season appearances induced by the treatments.

The results of these, and of numerous other experiments conducted by the Department, have shown the importance of the incidence of the spring rainfall and late frosts in relation to the time of heading and flowering of wheat, in determining both the apparent severity of root-rot lesions and the yield of the crop. It is a well established fact that plants at the flowering stage are conspicuously sensitive to drought (Loehwing 1940).

TABLE 9.—FIELD MINERAL TREATMENT EXPERIMENTS, NHILL, 1938-40, SHOWING COMPOSITIONS IN POUNDS PER ACRE OF MINERAL MIXTURES USED IN EACH SEASON.

—	Mixture Number.													
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
1938.														
Superphosphate ..	112	112	112	112	112	112	..	..	..	..	..	..	..	..
Sulphate of ammonia	..	56	56	56	56	56	..	..	..	..	..	..	..	..
Zinc sulphate ..	15	15	15	15	15	15	..	..	..	..	..	..	..	..
Manganese sulphate ..	20	..	20	20	..	..	..	..	..	..	..	..	..	..
Magnesium sulphate	30	30	30	30	..	..	..	..	..	..	..	..	..	..
Copper sulphate ..	..	..	..	2	4	..	..	..	..	..	..	..	..	..
Borax	..	..	..	0·5	..	..	..	..	..	..	..	..	..	..
Cobalt chloride ..	..	..	..	..	..	4	..	..	..	..	..	..	..	..
1939.														
Superphosphate ..	112	112	112	112	84	84	84	84	84	84	112	..	..	..
Sulphate of ammonia	37	37	56	56	28	28	28	28	28	28	56	..	..	..
Zinc sulphate ..	10	10	10	15	10	10	10	10	10	10	..	..	..	..
Manganese sulphate ..	10	10	10	20	10	10	10	10	10	10	20	..	..	..
Magnesium sulphate	10	10	10	30	..	10	10	10	10	10	30	..	..	..
Copper sulphate ..	2	4	2	2	2	2	2	2	2	2	2	..	..	..
Borax	0·5	1	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	..	..	..
Iron sulphate ..	..	..	..	..	..	..	10	..	10	10	..	..	..	..
Cobalt chloride ..	..	..	..	..	..	..	..	2	2	2	..	..	..	..
Potassium sulphate ..	..	..	..	..	..	..	..	..	..	10	..	..	..	..
Sodium iodide ..	..	..	..	..	..	..	..	..	..	0·5	..	..	..	..
Potassium bichromate	..	..	..	..	..	..	..	..	..	0·5	..	..	..	..
Nickel chloride ..	..	..	..	..	..	..	..	..	..	0·5	..	..	..	..
Ammonium molybdate	..	..	..	..	..	..	..	..	..	0·5	..	..	..	..
Barium chloride ..	..	..	..	..	..	..	..	..	..	0·5	..	..	..	..
Tin chloride ..	..	..	..	..	..	..	..	..	..	0·5	..	..	..	..
1940.														
Superphosphate ..	112	112	112	112	112	112	112	112	112	56	56	56	56	56
Sulphate of ammonia	..	28	28	28	28	28	28	56	56	56	56	56	56	56
Zinc sulphate ..	10	10	10	10	10	10	10	10	10	..	10	..	10	10
Manganese sulphate ..	10	10	..	10	10	..	10	10	10	10	10	10	..	10
Potassium sulphate ..	15	..	15	15	15	..	15	10	10	15	15	15	15	10
Copper sulphate ..	..	..	..	..	3	..	3	3	3	..	..	3	..	3
Iron sulphate ..	..	..	..	..	..	10	10	10	10	..	..	..	..	10
Magnesium sulphate	..	..	..	..	..	..	10	10	10	..	..	..	..	10
Cobalt chloride ..	..	..	..	..	..	..	..	..	2	..	..	..	..	..
Ammonium molybdate	..	..	..	..	..	..	..	..	2	..	..	..	..	..
Nickel sulphate ..	..	..	..	..	..	..	..	..	2	..	..	..	..	..

In some seasons, the application of superphosphate to wheat on Wimmera black ground has increased the yield by as much as 100 per cent., yet in other seasons this treatment has had no significant effect on yield. The importance of weather conditions on the response of wheat to fertilizers has also been recognized by Russell and Sallans (1940) in their work on common root-rot.

The high yields obtained in 1940 on black fallow soil when the yearly rainfall was approximately 10 inches (half normal) of which only 5½ inches fell during the growing period, are attributable to the conservation of soil moisture by sound cultural practices during the fallow period preceding sowing.

Table 10.—Results of Mineral Treatment Experiments with Ghurka Wheat on Black Fallow Soil, at Nhill, During the Seasons 1938 to 1940, Showing the Effects of Treatments on the Root-rot Index and Yield.

Treatment per Acre.	1938.		1939.			1940.		
			Root Rot Index.			Root Rot Index.		
	Root Rot Index.	Yield, Bus./ac.	First Sample 20th August.	Second Sample 4th December.	Yield. Bus./ac.	First Sample 16th September.	Second Sample 9th December.	Yield Bus./ac.
Untreated	..	..	40·2	66·0	27·3	55·3	64·3	38·8
Superphosphate, 1 cwt.	45·3	26·4	43·1	54·2	26·6	44·8	53·9	43·0
Superphosphate, 1 cwt. + Zinc sulphate, 8 lb.	38·2	28·9	..	..	..	..	..	..
Superphosphate, 1 cwt. + Zinc sulphate, 10 lb.	..	..	39·2	48·4	30·5	48·0	53·4	46·1
Superphosphate, 1 cwt. + Zinc sulphate, 15 lb.	38·0	28·3	..	..	..	..	..	..
Superphosphate, 1 cwt. + Zinc sulphate, 30 lb.	39·3	28·0	..	..	..	..	..	..
Superphosphate, 1 cwt. + Brown Zinc dross, 15 lb.	37·9	27·5	..	..	..	..	..	..
Superphosphate, 1 cwt. + Sulphate ammonia, 37 lb.	..	..	38·2	53·5	28·4	..	..	..
Superphosphate, 1 cwt. + Sulphate ammonia, 37 lb + Zinc sulphate, 10 lb.	..	..	32·7	54·5	31·5	..	..	..
Superphosphate, 1 cwt. + Sulphate ammonia, 56 lb. + Zinc sulphate, 15 lb.	38·4	29·4	..	..	..	..	..	..
Superphosphate 1 cwt. + Sulphate ammonia 28 lb.	..	..	..	..	..	50·4	55·0	43·2
Superphosphate 1 cwt. + Sulphate ammonia 28 lb. + Zinc sulphate 10 lb.	..	..	..	..	..	47·1	58·1	42·3
Mineral mixture No. 1	40·4	27·6	34·4	54·6	28·8	48·6	57·8	42·1
Mineral Mixture No. 2	37·2	30·1	34·6	54·9	29·6	46·4	58·6	42·2
Mineral Mixture No. 3	36·5	30·3	33·3	56·7	29·0	46·7	57·9	43·0
Mineral Mixture No. 4	34·6	31·4	25·5	52·7	30·3	42·3	61·4	43·7
Mineral Mixture No. 5	38·5	28·4	39·8	51·0	28·9	49·1	60·7	45·1
Mineral Mixture No. 6	33·3	29·6	35·0	45·9	30·6	46·8	60·6	45·7
Mineral Mixture No. 7	..	..	31·0	52·0	30·8	46·9	52·1	43·5
Mineral Mixture No. 8	..	..	33·6	52·6	29·2	49·0	57·8	43·5
Mineral Mixture No. 9	..	..	33·3	47·8	28·1	53·5	57·5	43·0
Mineral Mixture No. 10	..	..	34·6	46·1	29·1	..	..	..
Difference for significance ..	3·9	2·4	6·3	13·2	2·3	5·7	17·9	4·0

Note.—For compositions of the mineral mixtures used in each season, see Table 9.

Wheat—Black Fallow Soil.

With regard to the wheat experiments on black fallow ground, it will be seen from Table 10 that certain of the treatments significantly decreased the severity of root-rot lesions and increased the yield, although as stated above the increases in yield were not as great as the early appearances of the plots would indicate. Owing to the adverse seasonal conditions experienced, the differences in yield due to applications of zinc sulphate in 1938, 1939, and 1940 were not comparable with those obtained in the previous two seasons (Millikan 1938). Although all the mineral mixtures produced an improvement in growth over plants treated with superphosphate or superphosphate plus sulphate of ammonia only, the plants receiving the No. 4 mixture in 1938 and 1939 were outstanding in regard to both height and depth of colour, particularly during the early parts of both seasons.

The No. 4 mixture was also more effective than the treatment superphosphate plus sulphate of ammonia plus zinc sulphate, thus confirming the results of the greenhouse experiments to the effect that some minerals other than zinc are important in inducing improvements in growth on Wimmera black soil (Table 8). The latter treatment was superior to either superphosphate plus sulphate of ammonia or superphosphate plus zinc sulphate, a result similar to that of Vanterpool (1940) in connection with browning root-rot of cereals. He found that nitrogenous fertilizers were of little or no value when applied singly, but when the phosphorus deficiency had been rectified, nitrogenous applications gave a further beneficial response. This is of particular interest as browning root-rot associated with *Pythium sp.* occurs in Wimmera black soil.

During the stage when marked differences in growth were apparent, the severity of root-rot lesions on the plants showing marked responses to the mineral treatments was significantly reduced. Samuel (1934) has made a similar observation in connection with the percentage of "take-all" infestation on manurial plots at the Waite Institute. High yield was associated with a small amount of take-all and vice versa.

The severity of lesions is evidently related to factors affecting the vigour of the plant; mineral treatment cannot make up for an inadequate rainfall, particularly immediately preceding and after heading. Such an inadequacy will seriously affect plant vigour and increase the apparent severity of root-rot damage. The deleterious effects of continued dry weather was relatively greatest in plants receiving mineral treatments which, earlier in the season, showed the greatest growth, and therefore had a greater normal water requirement than untreated plants. For this reason, treatments which were accompanied by a significant decrease in the root-rot index at the time of the first sampling before heading, produced no such change at the time of the second sampling shortly before harvest.

At this stage, when the grain was practically dead ripe and the roots were becoming senile, the root-rot index was much higher than that at the time of the first sample just prior to heading. It was considered unlikely, however, that this increase in the root-rot index at such a late stage in the life of the plant would, in itself, have any significant effect on yield.

A large number of lesions on the wheat roots were cultured at each sampling to determine the relative abundance of the fungi associated with them.

The following are the fungi which were isolated, in order of their relative abundance:—

1938—

Helminthosporium sativum, Fusarium sp., Fusarium culmorum, Dendryphium sp. (Plate XI., fig. 10), *Ophiobolus graminis, Curvularia ramosa* and *Pythium sp.*

Eelworms (*Heterodera schachtii* and *Pratylenchus pratensis*) occurred to a limited extent.

1939—

First Sampling, 20th August.

Pythium sp., Phoma spp., Stemphylium lanuginosum, Helminthosporium sativum, Curvularia ramosa, Fusarium culmorum, and *Dendryphium sp.*

Second Sampling, 4th December.

Fusarium culmorum, Dendryphium sp., Fusarium sp., Periconia circinata, Curvularia ramosa, Macrosporium sp., Fusarium moniliforme, var. subglutinans; Fusarium scirpi, var. compactum; Helminthosporium sativum; Ophiobolus graminis, Rhizoctonia solani, and *Wojnowicia graminis.*

Eelworms (*Heterodera schachtii* and *Pratylenchus pratensis*) also occurred to a limited extent.

1940—

First Sampling, 16th September.

Pythium sp., Fusarium sp., Fusarium culmorum, Dendryphium sp., Ophiobolus graminis, Helminthosporium sativum, Fusarium scirpi, var. compoctum, Fusarium sp., Stemphylium lanuginosum, Rhizoctonia solani, and *Periconia circinata.*

Second Sampling, 9th December.

Pythium sp., Fusarium sp., Dendryphium sp., Fusarium culmorum, Periconia circinata, Sclerotium sp., Helminthosporium sativum, Fusarium scirpi, var. compactum, Ophiobolus graminis, Alternaria sp., Rhizoctonia solani, Fusarium moniliforme, var. subglutinans, Stemphylium lanuginosum, and an undetermined sterile fungus.

Eelworms also occurred commonly.

It will be seen that a complex succession of fungi was associated with the root-rot lesions. Simmonds and Ledingham (1937), Sprague (1938), and others have similarly reported that cereals may be attacked by a complex of soil-borne fungi, while Garrett (1936), Lal (1939) and others have found that a similar sequence of organisms to that reported above occurred in wheat plants affected with take-all following the invasion of the root by the primary parasite.

Entry of the fungi into the roots may be facilitated by eelworm attack or by the breaking, at more or less regular intervals, of the tissues of the young roots outside the vascular areas which has been frequently found to occur in wheat plants growing on Nhill black soil (Plate XI., fig. 9); similar symptoms have been observed on the sub-crown internode. The cause of this phenomenon is at present unknown; it may, however, be related to the fact that this soil expands and contracts appreciably with changes in its moisture content.

It is also of interest to note that *Periconia circinata* also occurred among Glynne's (1939) isolations from wheat at Rothamstead.

The fungus, tentatively referred to *Dendryphium,* is believed to be previously unrecorded in pathological history. On potato-dextrose-agar it produces dense, dark olive green to black colonies with a lighter coloured aerial growth. Large chlamydospores of very variable shape occur freely, while conidia are produced very sparingly on the aerial mycelium. The latter are ovoid, dark, 4 to 9 celled, sometimes branched. $15\text{-}27\mu \times 7\text{-}10\mu$, acrogenous, usually solitary, very occasionally catenate on short geniculate conidiophores (Plate XI., fig. 10).

Wheat—Red Fallow Soil.—The Wimmera red soils are lighter in texture, and poorer in some mineral nutrients, lime and organic matter than the black soil (Table 5). Their pH is in the vicinity of 6·5 to 7·0 as against about 8·3 for the black type. Although lighter, these red soils are not as easily worked as the black. They dry out readily, tend to set, and do not show the self-mulching properties of the black soils.

None of the treatments tested (Table 11) produced the same relative improvement in growth as was obtained on black ground, indicating that the red soil presents a problem different from that of the black ground. It is evident that the mineral treatments applied to date have not been suitable to its particular requirements. This confirms the results of the greenhouse experiments shown in Tables 5 and 8. The results of analyses in Table 5 show some of the important differences in the mineral composition of plants grown on red and black soils respectively. In Western Australia Teakle and Thomas (1939) have had a similar experience. They found that mineral mixtures significantly depressed the yields of wheat on various soil types, although in one instance the mixture produced a deeper green colour in the early stages of growth.

In 1939 a significant reduction in the root-rot index and a significant increase in yield was produced by a dressing of superphosphate 84 lb. + sulphate of ammonia 28 lb. + zinc sulphate 10 lb. per acre.

In contrast with the experience in 1939, much higher root-rot indexes and lower yields were obtained from the red fallow plots than from those on black fallow ground in 1940. This is attributable to the much lower incidence of rainfall in the latter year, which obviously affected the plants on the light red soils to a much greater degree than those on the heavier, more moisture retentive, black soil. It was estimated that in 1940, nearly 50 per cent. of the plants grown on the red soil produced "dead heads", and the grain in the remainder was very light and pinched.

TABLE 11.—RESULTS OF MINERAL TREATMENT EXPERIMENTS WITH GHURKA WHEAT ON RED FALLOW SOIL AT NHILL DURING 1939 AND 1940, SHOWING EFFECTS OF TREATMENTS ON THE ROOT-ROT INDEX AND YIELD.

Treatment per Acre.	Root Rot Index.	Yield Bus./ac.
1939.	3rd November.	
Superphosphate 84 lb.	63·1	20·6
Superphosphate 84 lb. + Zinc sulphate 10 lb.	60·8	19·8
Superphosphate 84 lb. + Sulphate ammonia 28 lb.	66·9	19·2
Superphosphate 84 lb. + Sulphate ammonia 28 lb. + Zinc sulphate 10 lb.	54·7	24·0
Mineral Mixture No. 6	58·7	20·5
Mineral Mixture No. 9	59·7	22·2
Difference for significance	4·5	3·9
1940.	27th November.	
Untreated	80·4	9·6
Superphosphate 1 cwt.	89·2	11·8
Superphosphate 1 cwt. + Zinc sulphate 10 lb.	93·4	10·9
Superphosphate 1 cwt. + sulphate ammonia 28 lb.	92·9	12·1
Superphosphate 1 cwt. + sulphate ammonia 28 lb. + Zinc sulphate 10 lb.	82·6	10·7
Superphosphate 1 cwt. + Sulphate ammonia 28 lb. + copper sulphate 5 lb.	84·2	11·0
Superphosphate 1 cwt. + Sulphate ammonia 28 lb. + Manganese sulphate 10 lb.	90·3	9·3
Superphosphate 1 cwt. + Sulphate ammonia 28 lb. + Iron sulphate 10 lb.	92·1	11·0
Mineral Mixture No. 7	90·1	11·5
Difference for significance	7·7	0·7

NOTE.—For compositions of mineral mixtures used in each season, see Table 9.

The lower root-rot index of the untreated plants in 1940 was due to the fact that at the time of sampling, which was very late in the season, these plants were much more immature than those receiving the remaining treatments.

The following fungi, in order of their relative abundance, were found to be associated with lesions on the roots of the wheat plants grown on red soil:—

1939—

Fusarium culmorum, Fusarium moniliforme, Fusarium scirpi, var. compactum, Sclerotium sp., Ophiobolus graminis, Fusarium sp., Phoma sp., Curvularia ramosa, Periconia circinata, Alternaria sp., and an undetermined fungus.

1940—

Fusarium culmorum, Fusarium scirpi, var. compactum, Fusarium moniliforme, Helminthosporium sativum, Fusarium sp., Curvularia ramosa, Helminthosporium sp., Sclerotium sp., Ophiobolus graminis and *Phoma sp.*

Over 80 per cent. of the isolations consisted of either one of the first three *Fusarium* species listed above.

Oats—Black Wheat Stubble Soil.—Oat experiments on black wheat stubble soil were conducted during the seasons 1938-1940 inclusive; the varieties of oats used were:—1938 and 1939 Mulga, 1940 Gidgee. Severe drought conditions were experienced in 1938, and the majority of the oat plants died before reaching maturity. Observations indicated, however, that treatment with superphosphate 1 cwt. + sulphate of ammonia 56 lb. + zinc sulphate 15 lb. per acre, and also mineral mixture No. 4 (see Table 9 for composition) produced an appreciable improvement in growth in the early stages of development. No response was observed from applications of superphosphate + zinc sulphate.

Details of the treatments sown in 1939 and 1940 are shown in Table 12.

TABLE 12.—RESULTS OF MINERAL TREATMENT EXPERIMENTS WITH OATS GROWN ON BLACK WHEAT STUBBLE SOIL AT NHILL DURING 1939 AND 1940, SHOWING THE EFFECTS OF TREATMENTS ON THE ROOT-ROT INDEX AND YIELD.

Treatment per Acre.	1939.		1940.*
	Root Rot Index. 2nd October.	Hay Yield. cwt./ac.	Grain Yield. bus./ac.
Untreated	22·8	28·4	11·9
Superphosphate ½ cwt.	..	..	12·3
Superphosphate 1 cwt.	22·0	28·4	..
Superphosphate 1 cwt. + Zinc sulphate 10 lb.	23·1	29·8	..
Superphosphate 1 cwt. + Zinc sulphate 40 lb.	20·0	29·6	..
Superphosphate 1 cwt. + Sulphate ammonia 37 lb.	23·3	33·5	..
Superphosphate ½ cwt. + Sulphate ammonia 56 lb.	..	..	13·1
Superphosphate 1 cwt. + Sulphate ammonia 37 lb. + Zinc sulphate 10 lb.	19·4	32·7	..
Superphosphate ½ cwt. + Sulphate ammonia 56 lb. + Zinc sulphate 10 lb.	..	..	13·5
Superphosphate ½ cwt. + Sulphate ammonia 56 lb. + Copper sulphate 5 lb.	..	..	13·3
Superphosphate ½ cwt. + Sulphate ammonia 56 lb. + Manganese sulphate 10 lb.	..	..	12·7
Mineral Mixture No. 1	16·3	33·4	..
Mineral Mixture No. 4	16·8	37·9	..
Mineral Mixture No. 6	20·2	33·6	..
Mineral Mixture No. 8	..	..	14·1
Mineral Mixture No. 9	21·9	31·6	14·0
Mineral Mixture No. 10	19·7	30·6	13·7
Mineral Mixture No. 11	17·9	37·9	14·6
Mineral Mixture No. 12	..	..	14·8
Mineral Mixture No. 13	..	..	15·3
Mineral Mixture No. 14	..	..	15·6
Difference for significance	6·0	2·5	2·0

* In 1940 only a composite sample for disease observation was obtained at harvest. The root rot index of this sample was 43·1.

† For compositions of the mineral mixtures used in each season, see Table 9.

Relatively greater differences in growth were induced between these treatments (Plate XII., fig. 12) on stubble and similar treatments on fallow. These differences in growth were accompanied by significant decreases in the severity of root-rot lesions, and significant increases in the yield of hay or grain (Table 12).

The root-rot indices of the stubble oat samples were found to be considerably lower than those of the wheat plants grown on fallow. The significance of this is discussed below.

The following fungi, in order of their relative abundance, were found to be associated with lesions on the roots of oats obtained from these experiments:—

Dendryphium sp., Curvularia ramosa, Fusarium culmorum, Alternaria sp., and an undetermined sterile fungus.

Discussion.

The field mineral treatment experiments with wheat and oats afford confirmation of the results of the greenhouse mineral treatment experiments with Wimmera soil, in which, in some instances, improvements in growth approaching that produced by soil sterilization resulted from the application of certain mineral mixtures. In view of this result, it is obvious that the beneficial effect of soil sterilization on plant growth is not attributable solely to the destruction of soil-borne plant parasites, but rather, as has been demonstrated in an earlier section of this paper, to the increase in the availability of plant nutrients. Evidence was thus obtained that other elements in addition to zinc may be of importance in stimulating the growth of cereals on Wimmera black soil. The stimulation induced by the application of these elements was greater on stubble than on fallow soil.

A fact of great significance is that the relative improvement in growth at harvest, induced by the mineral mixtures, has been much greater under greenhouse than under field conditions. It was evident that the soil moisture content had a governing influence on responses resulting from the application of such mixtures. While adequate soil moisture was at all times available to the greenhouse plants, the field plants, particularly early in the springs of 1938 and 1940, suffered periods of severe drought, the effects of which on the plants was dependent entirely on the relative growth and degrees of maturity induced by the various treatments. As the mineral treated plants always showed the greatest initial growth and earlier maturity, it was they which were most severely affected by periods of drought. Loehwing (1940) has stated that plants are very conspicuously sensitive to drought during the flowering period.

Mineral treatment has been much more effective in improving the growth of cereals on black than on red ground. The latter soil differs in important respects from black soil, some of which are evidently limiting factors in relation to the responses induced by such treatments as have been applied to date; the treatments applied must obviously be such as to meet the particular needs of the soil concerned.

The growth of wheat and oats on black wheat stubble soil in the Wimmera district is normally much inferior to that obtained with either wheat or oats on fallow. It is of significance, therefore, that the stubble oat root-rot indexes shown in Table 12 are very much lower than those obtained in the wheat treatments on fallow (Table 10). Another important fact is that the relative responses resulting from the application of certain nutritional treatments to wheat or oats on stubble have been relatively much greater than those induced by similar treatments in wheat on fallow. These results indicate that the poor growth on stubble is associated more with the inability of the plant to obtain an adequate supply of certain nutrients from the stubble soil than to the effects of a severe infestation of parasitic root-rot fungi. It must be emphasized that this conclusion applies for wheat as well as oats grown on Wimmera black wheat stubble soil, as the former cereal was used exclusively in the greenhouse experiments. The presence of wheat straw obviously did not increase the pathogenicity of soil-borne pathogens to the extent that they became a serious limiting factor to the growth of wheat on wheat stubble. This conclusion appears to be in contrast to that of Tyner (1940), who found that in greenhouse pathogenicity tests with *Ophiobolus graminis* and *Helminthosporium sativum* the development of disease on the basal parts of wheat seedlings was greatest in the presence of wheat straw compost, and least in oat straw composts. He concluded that a greater biological control of the pathogens resulted from the activities of micro-organisms associated with the decomposition of oat straw than wheat straw.

The number of micro-organisms is much greater in cropped than in fallow soil (Waksman 1931, Starkey 1931, Timonin 1940A). Penman and Rountree (1932) also found this to be so under Victorian conditions. They confirmed Starkey's observations to the effect that the growth of the crop interfered with the accumulation of nitrate in the soil, the cropped soil (after allowances for the nitrate in the crop itself) containing less nitrate than adjoining fallow soil. It has been suggested (Russell 1927), that one of the chief causes of the depressed nitrate content of cropped soils is its utilization by the increased population of soil organisms.

To this utilization of nitrate by organisms decomposing the soil organic matter is attributable the depression of nitrates which is a characteristic feature of Victorian stubble soils. This large population similarly builds into its thallus the other soil nutrients which are essential for its growth. After a period of fallow, during which the completion of the decomposition of the crop debris occurs, these nutrients are again rendered available to the plant through the autolysis of the organisms. The effect of fallowing, in this regard, is therefore similar in nature though not in degree to that of soil sterilization. Starkey (1938) has found that fungus hyphae were abundant even in fallow soil. Therefore, the sterilization of such soils and the subsequent autolysis of these organisms renders further mineral nutrients available to the plant.

The above is considered to be the explanation of the much greater response obtained from the application of mineral mixtures to stubble than to fallow soils, and the increased availability of minerals and the relatively greater improvement in growth of stubble sown, as compared with fallow sown plants, which results from sterilization of these soils.

These conclusions suggest the concept that a soil organism may have a deleterious effect on plant growth as a result of its saprophytic existence in the rhizosphere. It would follow that this effect would be greatest where the available supply of any nutrients was normally close to the threshold value for satisfactory plant growth in that soil. References to specific instances in the literature, where the deleterious effect of soil saprophytic organisms has been demonstrated, were cited earlier in this paper. A very brief discussion of the possible effects of changes in the soil reaction on the competition for the soil minerals between the soil organisms and the plant was also made.

In view of these results, it is considered that the question of the effects of stubble on the occurrence of root-rot disease requires further examination, as it has often been assumed that ploughing in of stubble favours the development of the disease. The above experiments have shown, however, that the poor growth of plants on stubble may be due to a large degree, to factors other than the direct effects of parasitic fungi. Indeed, the antagonistic effects on root-rot fungi of the organisms associated with the decay of wheat stubble in the soil (Garrett 1934, Waksman 1937, Lal 1939, Sandford and Cormack 1940) may decrease very appreciably the virulence of root-rot fungi in such soil. On the other hand, this saprophytic flora may be the indirect cause of the poor growth of cereals on stubble soils due to its demands on the available soil minerals.

These conclusions may be important in areas such as the Victorian Mallee district where the ploughing in of wheat stubble is recommended in preference to " burning off " to help check the tendency of the soil to drift. Any immediate benefit that " burning off " may have over that of " ploughing in " of stubble may be due at least as much to the readily available mineral ash which is returned to the soil as a result of the former practice, as to any destruction of fungus inoculum. In this regard, the writer has often observed in the case of Wimmera black soils that a much better growth of stubble sown oats results on patches where the stubble straw has previously been raked into small heaps before burning.

It would seem from the results of these greenhouse and field experiments that deficiencies of, firstly, soil moisture, and secondly, available soil nutrients are of more importance than the direct effects of root-rot fungi in limiting the growth of cereals on Wimmera black soil under field conditions. Lack of adequate soil moisture has the greatest effect on the plant immediately prior to and during heading. Some of the principal effects of soil drought on the wheat plant have been discussed by Loehwing (1940) and Whiteside (1941).

There is no doubt that physiological disturbances in the plant, induced by adverse changes in the moisture and available nutrients of the soil, profoundly affect the apparent severity of root- and foot-rot disease. References relating to the relationship between mineral deficiencies and the disease have been cited earlier. It has been observed in Victoria that "dead heads" may be very prevalent in wheat grown on Wimmera red soil in seasons in which very dry weather is experienced. These soils are lighter in texture than the black and have a lower moisture holding capacity. Similar observations on the effects of dry weather have been made by Schaffnit (1930), Machacek and Greaney (1935), Hynes (1937), Shen (1940), and others. Under such conditions, the nutritional relationships of the plant are not good, and the direct effects of the associated fungi are of secondary importance when compared with the effects resulting from the occurrence of an unfavourable plant environment during a critical period in the development of the plant.

A somewhat similar conclusion has been arrived at by the Council for Scientific and Industrial Research (Anon. 1940). From experimental evidence it has expressed the tentative view that the "white-head" condition, which in the field often accompanies the presence of *Ophiobolus graminis* in the base of the wheat plant, is the expression of physiological trouble associated with the chemical composition of the soil. In this regard, it should be noted that striking results in the elimination of "dead heads" in wheat have been obtained by the application of copper sulphate to certain areas in Western Australia (Teakle et al 1941).

Such a condition is parallel to the occurrence of oat blast, which Johnson and Brown (1940) have demonstrated to be induced by any adverse influence on the normal nutritional condition of the oat plant, from the time the spikelets are initiated until just prior to the emergence of the panicle.

It has also been shown that adverse environmental factors immediately prior to and during heading may profoundly influence the response of cereals to fertilizer treatments.

The fact that the presence of parasitic fungi in the roots of cereals is often accompanied by more or less severe symptoms of disease in other parts of the plant does not necessarily mean, therefore, that such symptoms are the direct result of the presence of the parasite in the roots of the plant. It has been shown that in some instances at least, they are more the outward expression of physiological disturbances in the plant induced by an unfavourable environment, than the results of the attack of parasitic organisms. Where the environment is favourable for plant growth, the presence of the root parasite may not have any observable effect on the growth and yield of the plant. Such a case has been recorded by Samuel and Greaney (1937). They isolated *Fusarium culmorum* from the roots of very healthy wheat plants in crops in various parts of England. Some of the isolations when tested out in the greenhouse proved pathogenic.

Similarly, the writer has freely isolated *Fusarium culmorum, Helminthosporium sativum, Curvularia ramosa* and a number of other fungi from the roots of otherwise healthy plants, growing in one instance in a crop which yielded almost 60 bushels per acre.

As Samuel and Greaney have pointed out, much work has been directed towards the status of such fungi as parasites, and that only recently has attention been paid to their status as weak secondary parasites. A full knowledge of the predisposing factors encouraging parasitic action is obviously essential to facilitate the solution of the problem of the control of foot- and root-rot diseases of cereals. The compilation of Beeson (1941) has shown the important influence of soil type on the mineral composition of plants. This aspect, as it affects the root-rot problem, has received little attention from pathologists.

The results of other research work relevant to the above conclusions have been exhaustively reviewed by Garrard and Lochhead (1938), Garrett (1939), Sandford (1939), and Simmonds (1939).

Root-rot of cereals, as it occurs in the Wimmera district of Victoria, cannot be regarded simply as due to the attack of a single organism, but rather to a complex of organisms. It follows from the foregoing that unless the physical, chemical, and biological conditions of the soils were comparable, the results of greenhouse pathogenicity tests with a fungus selected from a complex of foot- or root-rot fungi, would have little or no relation to the effects of the same organism under field conditions.

Summary.

Using purified synthetic nutrient solutions, the elements manganese, copper, zinc, and iron were found to be essential for the growth of three of the root-rot fungi occurring in Wimmera black soil, namely, *Fusarium culmorum, Helminthosporium sativum,* and *Curvularia ramosa.*

Amino nitrogen improved the yields of *F. culmorum* and *C. ramosa,* but caused sectoring in *H. sativum.* The growth of the latter fungus was stimulated by the addition of vitamins B_1, C and nicotinic acid to the nutrient solution.

Steam sterilization of Wimmera black fallow soil resulted in a very appreciable improvement in the growth of wheat, but concomitantly exercised a deleterious effect on the response of the plants to the application of zinc sulphate. Analyses of the plants showed that this effect may actually be associated with an excess of zinc in the plant, as it was found that the calcium, potassium, phosphorus, manganese, zinc, copper and nitrogen in the soil had become more available to the plant as a result of the sterilization of the soil. The percentage of iron in the plants was reduced by sterilization. Similar changes in the availability of minerals resulted from the sterilization of Wimmera red fallow soil.

Steam sterilization had no effect on soil reaction. Formalin sterilization induced a similar or better improvement in growth to steam sterilization.

Comparative analyses of wheat plants grown on Wimmera black and red fallow soils respectively showed that on the latter type of soil the plants contained the highest phosphoric acid (P_2O_5), zinc and copper, and the lowest lime (CaO), potash (K_2O), iron and manganese percentages.

The zinc responsiveness of Wimmera black soil, which is destroyed by steam sterilization, can be re-established by inoculating such sterilized soil two months prior to sowing with bacteria normally occurring in unsterilized soil. The re-inoculation of sterilized black fallow soil with some of the fungi and bacteria normally present in unsterilized soil, or with 1 per cent. of unsterilized soil two months before sowing, had no significant effect on the mineral composition of the plants four weeks after germination, but decreased the percentages of phosphoric acid (P_2O_5), potash (K_2O), magnesia (MgO), zinc and manganese in the plant at eight weeks after germination.

At this time, however, the general nutritional level of the plants grown in the re-inoculated soil was still much higher than that of plants growing in unsterilized soil.

The increase in plant growth resulting from soil sterilization has been relatively much greater on Wimmera black wheat stubble soil than on comparable fallow soil. Similarly the responses to the application of mineral mixtures under both field and greenhouse conditions have been greater on black stubble soil than on fallow soil. During the growth of the plant up to the heading stage, the improvement induced by the addition of the mixtures to stubble soils under greenhouse conditions has in some instances been equal to or even better than that resulting from soil sterilization. After this stage the sterilized soil plants usually showed best growth.

It follows that the normally poor growth of oats or wheat obtained on Wimmera black stubble soils is attributable less to the occurrence of root-rot infection, than to the inability of the plant to obtain an adequate supply of nutrients from the stubble soil.

Whereas the inclusion of the elements magnesium, copper, cobalt, molybdenum, nickel and boron in the mixtures applied to black fallow soil significantly depressed the yield, their addition to the stubble soil mixtures caused an appreciable increase in the weight of dry matter produced. The process of fallowing Wimmera black soil evidently increases the availability of some at least of the elements listed above and renders undesirable the addition of further amounts of them to the soil.

These results suggest that soil-inhabiting organisms may effect the growth of the plant indirectly by using the soil nutrients for their own vital processes, thus reducing the amount available to the plant. The effects of this competition on plant growth would be most marked where the available supply of any particular nutrients normally approached the threshold value for satisfactory plant growth in that soil. This develops the concept that a soil organism may have a deleterious effect on plant growth because of its saprophytic existence in the rhizosphere.

Environment has an important effect on the nutritional requirements of the plant. Under greenhouse conditions the relative improvement in growth resulting from mineral treatment was greater than under field conditions where the incidence of rainfall in relation to heading was found to be of fundamental importance in determining the relative differences in yield induced by mineral treatment.

In the case of Wimmera red fallow soil the relative improvement in growth produced by the application of mineral mixtures was not as great as that obtained on black fallow soil, indicating that any mineral treatments applied must be such as to meet the particular needs of the soil concerned.

In the field, increased growth resulting from the application of mineral mixtures to wheat and oats on Wimmera black soil was accompanied by significant decreases in root-rot. Numerous fungi were associated with root-rot lesions on cereals in the Wimmera district of Victoria.

As the real effect of root-rot fungi on the growth of the plant may be considerably less than their apparent effect, it is of great importance to distinguish between symptoms caused by physiological disorders in the plant induced by unfavourable environmental factors and those caused by the direct effects of foot- and root-rot organisms. The alleviation of the physiological disorders will appreciably decrease the apparent severity of the foot- and root-rot diseases.

Acknowledgment.

This work was performed in the Biological Branch of the Department of Agriculture, Victoria. The writer wishes to acknowledge his indebtedness to the Agricultural Research Chemist, Mr. W. R. Jewell, for the chemical analyses, and to Mr. C. P. Dahlenburg, of Nhill, for untiring assistance in the conduct of the field experiments described in this report.

References.

Anon., 1940.—In Fourteenth Annual Report of the Council for Scientific and Industrial Research for the Year 1939-40, p. 13.

Ark, P. A., 1937.—Little Leaf or Rosette of Fruit Trees VII. Soil Microflora and Little Leaf or Rosette Disease. *Proc. Amer. Soc. Hort. Sci.,* xxxiv.: 216-221.

Arnon, D. I., and Stout, P. R., 1939.—The Essentiality of Certain Elements in Minute Quantity for Plants with special reference to Copper. *Plant. Physiol.,* 14: 371-375.

Beeson, K. C., 1941.—The mineral composition of crops with particular reference to the soils in which they were grown. A review and compilation. U.S.D.A. Misc. Publ. 369.

Blank, L. M., 1939.—The response of Phymatotrichum omnivorum to heavy metals and other elements. *Abs. in Phytopath.,* 29: 2.

———, 1941.—Response of Phymatotrichum omnivorum to certain trace elements. *Journ. Ag. Res.,* 62: 129-159.

Chandler, W. H., 1937.—Zinc as a nutrient for plants. *Bot. Gaz.,* xcviii.: 625-646.

Chapman, H. D., Liebig, G. F., and Parker, E. R., 1939.—Manganese studies: California Soils and Citrus Leaf Symptoms of Deficiency. *Calif. Citrogr.,* xxiv.: 427, 454; xxv.: 11, 15.

———, ———, and Vanselow, A. P., 1940.—Some nutritional relationships as revealed by a study of mineral deficiency and excess symptoms on citrus. *Soil Sci. Amer. Proc.,* 4: 196-200.

Christensen, J. J., and Davies, F. R., 1937.—Nature of Variation in Helminthosporium sativum. *Mycologia,* 29: 85-99.

Clark, F. E., 1940.—Notes on types of bacteria associated with plant roots. *Trans. Kansas Acad. Sci.,* 43: 75-84.

Conrad, J. P., 1939.—Retention of some phosphorus compounds by soils as shown by subsequent plant growth. *Jour. Ag. Res.,* 59: 507-518.

Eaton, F. M., and Wilcox, L. V., 1939.—The behaviour of boron in soils. U.S.D.A. Tech. Bull. 696.

Foster, J. W., 1939.—The heavy metal nutrition of fungi. *Bot. Rev.,* v.: 207-239.

Garrard, E. H., and Lochhead, A. G., 1938.—Relationships between soil micro-organisms and soil-borne plant pathogens. A Review *Sci. Agr.,* 18: 719-737.

Garrett, S. D., 1934.—Factors affecting the pathogenicity of cereal foot-rot fungi. *Biol. Rev.* 9: 351-361.

———, 1936.—Soil conditions and the take-all disease of wheat. *Ann. App. Biol.,* 23: 667-699.

———, 1939.—Soil-borne fungi and the control of root disease. *Imp. Bur. Soil Sci. Tech. Comm.* 38.

GERRETSEN, F. C., 1935.—The effect of manganese deficiency on oats, in relation to soil bacteria. *Trans. third int. Congr. Soil Sci.,* 1: 173-174. R.A.M., xv.: 356-358, 1936.

GLYNNE, MARY D., 1939.—Fungal invasion of the roots of healthy wheat plants. *Trans. Brit. Mycol. Soc.,* xxiii.: 210.

HEARMAN, J., 1938.—Minor elements and the pine. *Aust. Forest* 3: 24-27.

HIBBARD, P. L., 1936.—Availability of plant nutrients. *Soil Sci. Soc. Amer. Proc.* 1: 149-151.

———, 1940 (*a*).—A soil zinc survey in California. *Soil Sci.* 49: 63-72.

———, 1940 (*b*).—The chemical status of zinc in the soil with methods of analysis. Hilgardia 13: 1-29.

———, 1940 (*c*).—Accumulation of zinc on soil under long persistent vegetation. *Soil Sci.* 50: 53-55.

HOAGLAND, D. R., CHANDLER, W. H., and STOUT, P. R., 1937.—Little leaf or rosette of fruit trees, vi. Further experiments bearing on the cause of the disease. *Proc. Amer. Soc. Hort. Sci.,* xxxiv.: 210-212.

HYNES, H. J., 1937.—Some aspects of the root-rot problem in wheat. *J. Aust. Inst. Agric. Sci.,* 3: 212-218.

JOHNSON, T., and BROWN, A. M., 1940.—Studies on oat blast. *Sci. Ag.,* 20: 532-550.

KESSELL, S. L., and STOATE, T. N., 1936.—Plant nutrients and pine growth. *Aust. Forest* 1: 4-13.

LAL, A., 1939.—Interaction of soil micro-organisms with Ophiobolus graminis Sacc., the fungus causing the take-all disease of wheat. *Ann. App. Biol.,* xxvi.: 247-261.

LEEPER, G. W., 1935.—Manganese deficiency of cereals, plot experiments and a new hypothesis. *Proc. Roy. Soc. Vic.,* xlvii. (n.s.): 225-261.

———, 1940.—Experiments on manganese deficiency disease ("Grey Speck") of cereals. *Proc. Roy. Soc. Vic.,* lii. (n.s.): 138-152.

———, and SWABY, R. J., 1940.—The oxidation of manganous compounds by micro-organisms in the soil. *Soil Sci.* 49: 163-169.

LEWIS, R. W., 1938.—Ascorbic acid (Vitamin C.) in fungus extracts. *Mich. Acad. Sci. Arts and Letters.* Papers 24: 31-35.

LOCHHEAD, A. G., 1940.—Qualitative studies of soil micro-organisms iii. Influence of plant growth on the character of the bacterial flora. *Canad. Jour. Res.* 18, Sec. C: 42-55.

———, TIMONIN, M. I., and WEST, P. M., 1940.—The microflora of the rhizosphere in relation to resistance of plants to soil-borne pathogens. *Sci. Agric.,* xx.: 414-418.

LOEHWING, W. F., 1940.—Mineral nutrients in relation to flower development. *Science* 92: 517-520.

McCALLA, T. M., 1940.—Cation adsorption by bacteria. *Jour. Bact.,* 40: 23-32.

McGEORGE, W. T., 1939.—Factors influencing the availability of native soil phosphate and phosphate fertilizers in Arizona soils. *Arizona Sta. Tech. Bull.* 82: 293-331.

MACHACEK, J. E., and GREANEY, F. J., 1935.—Studies on the control of root-rot diseases of cereals caused by Fusarium culmorum and Helminthosporium sativum. *Sci. Agric.,* 15: 607-620.

MIDGLEY, A. R., and DUNKLEE, D. E., 1940.—The effect of lime on the fixation of borates in soil. *Soil Sci. Soc. Amer. Proc.,* 4: 302-307.

MILES, L. E., 1936.—Effect of potash fertilizers on cotton wilt. *Mississippi Sta. Tech. Bull.* 23.

MILLIKAN, C. R., 1938.—A preliminary note on the relation of zinc to disease in cereals. *Jour. Dept. Ag. Vic.*, 36: 409-416.

———, 1940.—Zinc requirement of wheat. The effect of superphosphate. *Jour. Dept. Ag. Vic.*, 38: 135-136.

MULDER, E. G., 1938 (*a*).—Over de betekenis var koper voor de groei van planten en micro-organismen, in het bijzonder een onderzock naar de oorzaak der ontginningsvjiekte. *Landb. Tijdschr. Wageningen*, 1: 898-902. R.A.M. xviii.: 240, 1939.

———, 1938 (*b*).—Sur l'influence du cuivre sur la croissance des micro-organismes. *Ann. Ferment*, n.s. iv.: 513-533. R.A.M. xviii.: 241, 1939.

PENMAN, F., and ROUNTREE, P. M., 1932.—Influence of a wheat crop on accumulation of soil nitrate. *Jour. Dept. Ag. Vic.*, 30: 496-504.

PERVUKHINA, N. V., 1938.—(Concerning the physiology of some species of Fusarium on wheat.) *Pl. Prot. Leningr.*, 16: 59-64. R.A.M. xviii.: 97, 1939.

PIPER, C. S., 1938.—The occurrence of "reclamation disease" in cereals in South Australia. C.S.I.R. pamphlet 78.

RICEMAN, D. S., and ANDERSON, A. J., 1941.—Response to zinc on a South Australian soil. *Jour. Aust. Inst. Ag. Sci.*, 7: 82.

ROBBINS, W. J., 1939.—Thiamin and symbiosis. *Bull. Torrey Bot. Club* 66: 569-572.

ROGERS, C. H., 1938.—Growth of Phymatotrichum omnivorum in solutions with varying amounts of certain mineral elements. *Amer. J. Bot.*, xxv.: 621-624.

RUSSELL, E. J., 1927.—Soil conditions and plant growth.

RUSSELL, R. C., and SALLANS, B. J., 1940.—The effect of phosphatic fertilizers on common root-rot. *Sci. Ag.*, xxi.: 44-51.

SADASIVAN, R. S., 1939.—Succession of fungi decomposing wheat straw in different soils, with special reference to Fusarium culmorum. *Ann. App. Biol.*, xxvi.: 497-508.

SAMUEL, G., 1934.—Report Waite Agricultural Research Institute 1925-32: 25-26.

———, and GREANEY, F. J., 1937.—Some observations on the occurrence of Fusarium culmorum on wheat. *Trans. Brit. Mycological Soc.*, xxi.: 114-117.

SANDFORD, G. B., 1933.—Some soil microbiological aspects of plant pathology. *Sci. Ag.*, 13: 638-641.

———, 1939.—Research on certain soil borne diseases as affected by other micro-organisms. *Sci. Agr.* xix.: 609-615.

———. 1941.—Studies in Rhizoctonia solani V. Virulence in steam sterilized and natural soil. *Canad. Jour. Res.*, 19: Sec. C, 1-8.

———, and CORMACK, M. W., 1940.—Variability in association effects of other soil fungi on the virulence of Helminthosporium sativum in wheat seedlings. *Canad. Jour. Res.* C, 18: 562-565.

SCHAFFNIT, E., 1930.—Ertragseinbussen im Getreidebau durch Fusskrankheiten. *Mitt. Dtsch. Landw. Ges.* 45: 247. R.A.M. ix.: 586, 1930.

SHEN, C. I., 1940.—Soil conditions and the Fusarium culmorum seedling blight of wheat. *Ann. App. Biol.* 27: 323-329.

SCHEUNERT, A., WAGNER, K. H., FINK, H., and KREBS, J., 1939.—(The synthesis of vitamin B_1 and B_2 (complex) by the yeast Torula utilis.) *Biochem. Ztschr.* 302: 1-11. E.S.R. 82: 750, 1940.

Simmonds, P. M., 1939.—A review of the investigations conducted in Western Canada on root-rot of cereals. *Sci. Agr.* 19: 565-582.

———, and Ledingham, R. J., 1937.—A study of the fungus flora of wheat roots. *Sci. Ag.* 18: 49-59.

Sprague, R., 1938.—Soil-borne diseases in coastal Oregon. *North West Sci.* 12: 74-80.

Starkey, R. L., 1931.—Some influences of the development of higher plants upon the micro-organisms in the soil IV. The influence of proximity to roots on abundance and activity of micro-organisms V. Effects of plants upon distribution of nitrates. *Soil Sci.* 32: 367-393, 395-404.

———, 1938.—Some influences of the development of higher plants upon the micro-organisms in the soil VI. Microscopic examination of the rhizosphere. *Soil Sci.* 45: 207-248.

Steinberg, R. A., 1935.—Nutrient solution purification for removal of heavy metals in deficiency investigations with Aspergillus niger. *Jour. Ag. Res.* 51: 413-424.

———, 1936.—Relation of accessory growth substances to heavy metals including Molybdenum, in the nutrition of Aspergillus niger. *Jour. Ag. Res.* 52: 439-448.

———, 1938.—The essentiality of gallium to growth and reproduction of Aspergillus niger. *Jour. Ag. Res.* 57: 569-574.

———, and Thom, C., 1940.—Mutations and reversions in reproductivity of Aspergilli with nitrite, colchicine and d-lysine. *Natl. Acad. Sci. Proc.* 26: 363-366.

Stout, P. R., and Arnon, D. I., 1939.—Experimental methods for the study of the rolé of copper, manganese and zinc in the nutrition of higher plants. *Amer. Jour. Bot.* 26: 144-149.

Teakle, L. J. H., and Thomas, I., 1939.—The effect of "minor" elements on the growth of wheat in other parts of the State. *Jour. Dept. Ag. W.A.* 16: 143-147.

———, ———, and Turton, A. G., 1941.—Experiments with micro-elements for the growth of crops in Western Australia. 1. Experiments in the Wheat Belt with Cereals. *Jour. Dept. Ag. W. Aust.*, Ser. 2, xviii.: 70-86.

Timonin, M. I., 1940 (*a*).—The interaction of higher plants and soil micro-organisms I. Microbial population of rhizosphere of seedlings of certain cultivated plants. *Canad. Jour. Res.* 18, Sec. C: 307-317.

———, 1940 (*b*).—The interaction of higher plants and soil micro-organisms II. Study of the microbial population of the rhizosphere in relation to resistance of plants to soil-borne diseases. *Canad. Jour. Res.* C, 18: 444-455.

Tyner, L. E., 1940.—The effect of crop debris on the pathogenicity of cereal root-rotting fungi. *Canad. Jour. Res.* 18, Sec. C: 289-306.

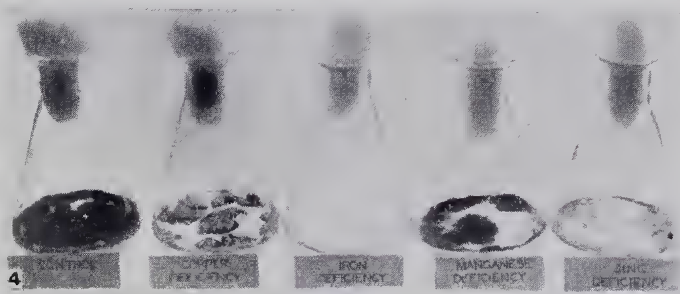
Vanterpool, T. C., 1940.—Present knowledge of browning root-rot of wheat with special reference to its control. *Sci. Agr.* xx.: 735-749.

Waite Institute, 1939.—Report of the Waite Agricultural Research Institute, S. Aust., 1937-1938: 53-55.

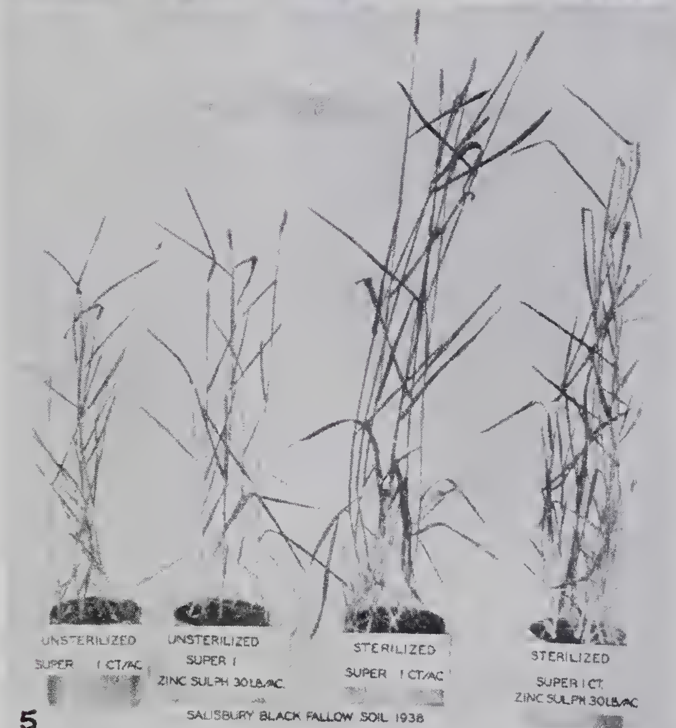
Waksman, S. A., 1931.—Principals of soil microbiology. Second edition, London. Balliere, Tindall, and Cox.

———, 1937.—Associative and antagonistic effects of micro-organisms. *Soil Sci.* 43: 51-68.

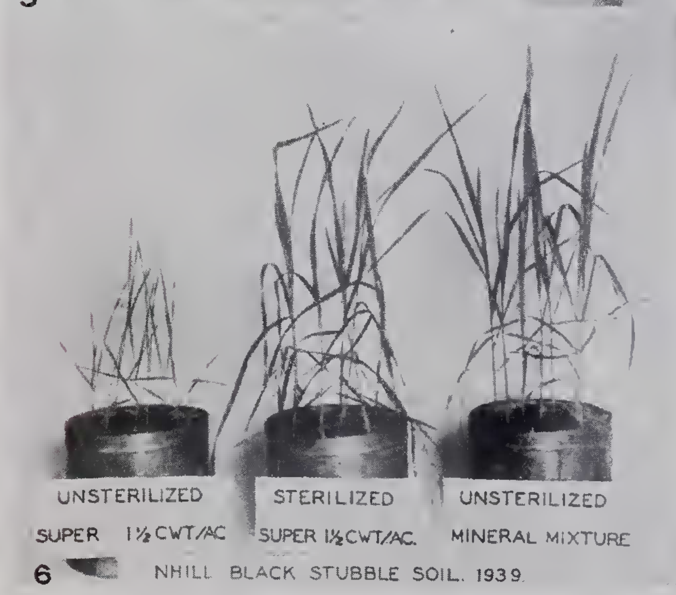
———, and Foster, J. W., 1938.—Effet du zinc sur la vegetation de Rhizopus nigricans et la production d'acide par cet organisme. *C.R. Acad. Sci.*, ccvii.: 483-486.


CONTROL
COPPER DEFICIENCY
IRON DEFICIENCY
MANGANESE DEFICIENCY
ZINC DEFICIENCY

4

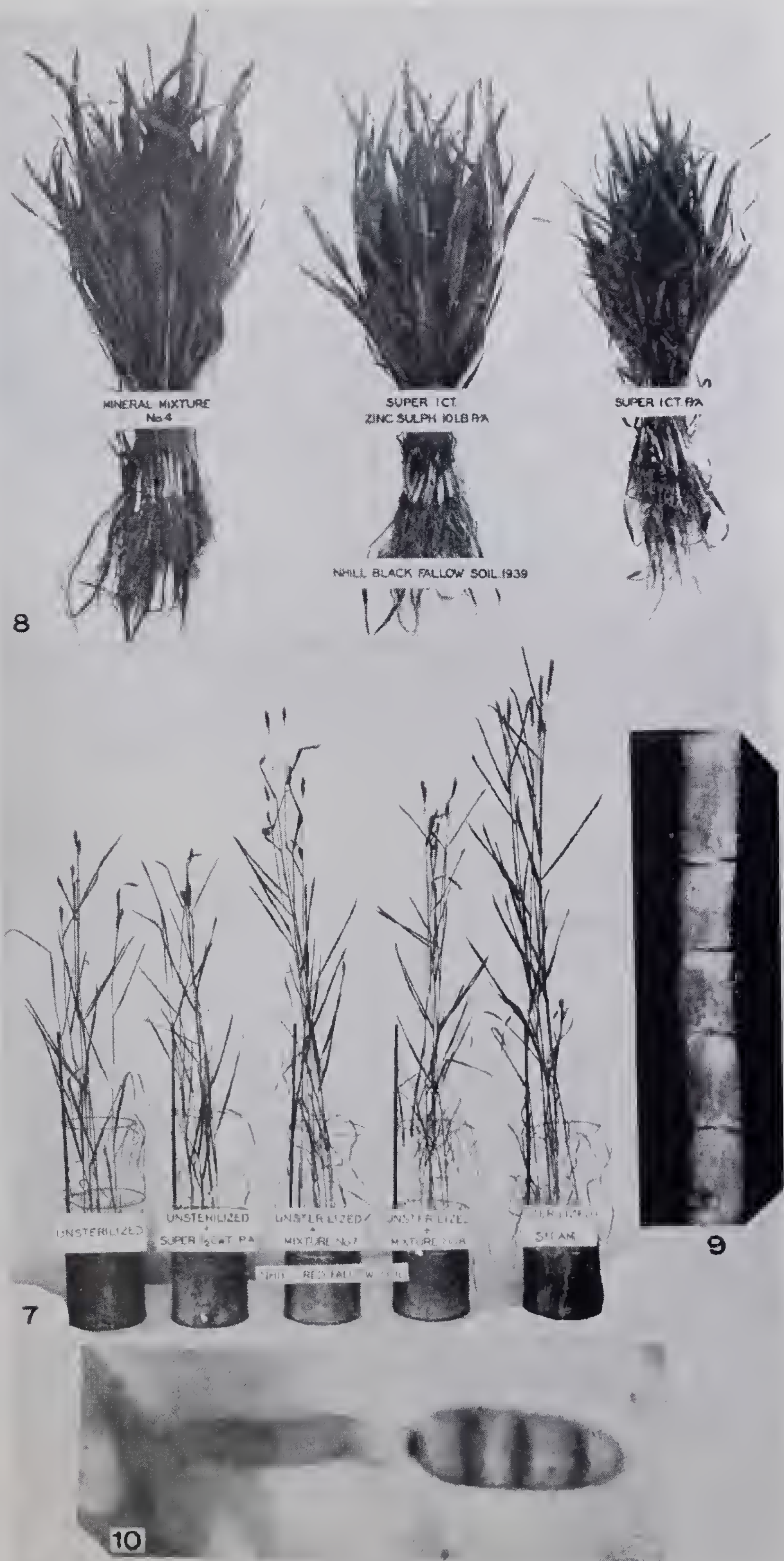

UNSTERILIZED
SUPER 1 CT/AC
UNSTERILIZED
SUPER 1
ZINC SULPH 30 LB/AC.
STERILIZED
SUPER 1 CT/AC
STERILIZED
SUPER 1 CT.
ZINC SULPH 30 LB/AC
SALISBURY BLACK FALLOW SOIL 1938

5


UNSTERILIZED
SUPER 1½ CWT/AC
STERILIZED
SUPER 1½ CWT/AC.
UNSTERILIZED
MINERAL MIXTURE
NHILL BLACK STUBBLE SOIL. 1939.

6

5089/42.


MINERAL MIXTURE
No.4
SUPER 1CT.
ZINC SULPH 10LB P/A
SUPER 1CT. P/A
NHILL BLACK FALLOW SOIL 1939
8
UNSTERILIZED
UNSTERILIZED
+
SUPER ½ CWT P/A
7
9
10

5089/42.

11

12

WALKER, J. C., and MUSBACH, F. L., 1939.—Effect of moisture, fertility and fertilizer placement on root-rot of canning peas in Wisconsin. *Jour. Ag. Res.* 59: 579-590.

WEST, P. M., and LOCHHEAD, A. G., 1940.—Qualitative studies of soil micro-organisms IV. The Rhizosphere in relation to the nutritive requirements of soil bacteria. *Canad. Jour. Res.* 18, Sec. C: 129-135.

WHITE, N. H., 1941.—Physiological studies of the fungus Ophiobolus graminis Sacc. 1. Growth factor requirements. *Jour. C.S.I.R.* 14: 137-146.

WHITESIDE, A. G. O., 1941.—Effect of soil drought on wheat plants. *Sci. Ag.*, xxi.: 320-334.

YOUNG, H. C., and BENNETT, C. W., 1922.—Growth of some parasitic fungi in synthetic culture media. *Amer. Jour. Bot.*, ix.: 459-469.

Explanation of Plates.

PLATE X.

FIG. 4.—Mineral deficiency experiment with *Curvularia ramosa*, showing the appearance of the cultures after incubation for six days at 25°C.

FIG. 5.—Showing the effects of soil sterilization and treatment with zinc sulphate respectively on the development of wheat grown in Wimmera black fallow soil at Burnley during 1938. Note improved growth due to soil sterilization. Maturity stimulated by the zinc sulphate application to unsterilized soil and retarded by a similar treatment to sterilized soil.

FIG. 6.—Showing comparative effects of soil sterilization and mineral treatment on growth of wheat on Wimmera black wheat stubble soil at Burnley during 1939. Photographed nine weeks after germination.

PLATE XI.

FIG. 7.—Showing the comparative effects of soil sterilization and mineral treatment on the growth of wheat on Wimmera red fallow soil at Burnley during 1940.

FIG. 8.—Representative samples of plants obtained 12 weeks after germination from wheat experiment on black fallow soil at Nhill during 1939. Note very improved growth of the mineral mixture at this stage.

FIG. 9.—Rootlet from wheat plant grown in Nhill black soil showing breaking at regular intervals of tissue outside the vascular area.

FIG. 10.—Conidiophore and spore of a previously undescribed fungus, tentatively referred to the genus *Dendryphium*, which is commonly associated with root-rot lesions on cereals grown in Wimmera black soil.

PLATE XII.

FIG. 11.—"Dead heads" in wheat growing on Wimmera red fallow soil.

FIG. 12.—Representative samples of plants obtained 18 weeks after germination from the oat experiment sown at Nhill in 1939 on black wheat stubble soil. Treatments as indicated. Note the improvement in growth induced by Mineral Mixture No. 4.